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A New Species of *Anagrus* (Hymenoptera: Mymaridae) from Tahiti, with Notes on Egg Parasitoids of Proconiine Sharpshooters (Hemiptera: Cicadellidae: Proconiini) in the World

Serguei V. Triapitsyn[1,*]

Abstract

Triapitsyn, S. V. 2013. A new species of *Anagrus* (Hymenoptera: Mymaridae) from Tahiti, with notes on egg parasitoids of proconiine sharpshooters (Hemiptera: Cicadellidae: Proconiini) in the world. J. Taiwan Agric. Res. 62(4):289–299.

One new fairyfly (Hymenoptera: Mymaridae) species, *Anagrus* (*Anagrus*) *iti* sp. n., described from Tahiti Island, French Polynesia, was reared from eggs of the invasive glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), which is native to southeastern USA and northeastern Mexico. An overview of the taxonomic and biological studies on the egg parasitoids (Hymenoptera: Aphelinidae, Mymaridae, and Trichogrammatidae) of various proconiine sharpshooters (Cicadellidae: Cicadellinae: Proconiini) in the New World is given. Of particular importance are the natural enemies of *H. vitripennis*, a notorious vector of the phytopathogenic bacterium, *Xylella fastidiosa*; with reference to its egg parasitoids and biological control in the non-native range are also provided.

Key words: Leafhopper, Proconiini, Egg parasitoid, *Anagrus*, Biological control.

INTRODUCTION

Proconiine sharpshooters belong to the New World cicadelline leafhopper tribe Proconiini (Hemiptera: Cicadellidae: Cicadellinae). The most notorious species is the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar 1821), a vector of plant diseases caused by the phytopathogenic bacterium, *Xylella fastidiosa* (Blua *et al.* 1999). The glassy-winged sharpshooter is a self-introduced pest in California from the southeastern USA where it is native; it is also native to northeastern Mexico (Triapitsyn & Phillips 2000). Turner & Pollard (1959) provided a brief overview of proconiine sharpshooter egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) in Georgia, USA. For a long time this was the only available publication on this group of natural enemies, which more recently has been shown to be amazingly diverse; almost every sharpshooter species has been found, upon closer examination, to have a complex of associated egg parasitoids. Establishment of *H. vitripennis* in California in the 1990s, later in the Hawaiian Islands and French Polynesia, and even more recently in Easter Island (Pilkington *et al.* 2005) prompted interest in proconiine sharpshooter investigations, including stud-

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ies of their egg parasitoids in North America (Triapitsyn & Phillips 1996, 2000; Triapitsyn *et al.* 1998; Triapitsyn, Bezark *et al.* 2002; Triapitsyn, Hoddle *et al.* 2002), mainly for classical biological control purposes (Morgan *et al*. 2000, 2002; Triapitsyn & Hoddle 2001, 2002; Pilkington *et al*. 2004, 2005).

Grandgirard *et al.* (2007) conducted a thorough survey of the native egg parasitoids of the invasive *H. vitripennis* in French Polynesia; among them was an undescribed *Anagrus* sp. (Hymenoptera: Mymaridae) which was reared in Tahiti Island from an egg mass of the glassy-winged sharpshooter. It is described here as a new species, *A. iti*.

Due to the recent identification of *X. fastidiosa* in the vineyards of Taiwan (Su *et al.* 2013), which causes Pierce's disease of grapevines (the first such record in Asia), a summary of the known egg parasitoids in several families of Chalcidoidea (Hymenoptera) of the proconiine sharpshooter leafhoppers (its vectors) in the world is also given. Information on egg parasitoids of the leafhoppers from the tribe Cicadellini, also from the subfamily Cicadellinae, is scarce globally; these will be reviewed separately. Cicadellini contain several known vector species of *X. fastidiosa* in the USA and elsewhere; particularly, *Kolla paulula* (Walker 1858) is a potential vector of Pierce's disease of grapevine and a common cicadelline species in Taiwan (Shih *et al.* 2009; Su *et al.* 2013).

TAXONOMY: DESCRIPTION OF A NEW SPECIES OF MYMARIDAE

Anagrus (*Anagrus*) *iti* Triapitsyn, sp. n.

Anagrus sp.: Grandgirard *et al*. 2007: 183 (an undescribed species)

Type material. Holotype female on slide, deposited in the Entomology Research Museum, University of California, Riverside, California, USA (UCRC), labeled: (1) 'FRENCH POLYNESIA: Society Islands, Tahiti I.[sland], Tapahi Point, 11.i.2005, J. Grandgirard, reared from eggs of Glassy-winged Sharpshooter, *Homalodisca coagulata*, on hibiscus'; (2) 'Mounted at UCR/ERM by V. V. Berezovskiy 2005 in Canada balsam' (UCRC ENT 265298). Paratypes: 16 females and 4 males on points, and 1 female and 1 male on slides (UCRC), same data as the holotype.

Description. Female. Body length (dry-mounted paratypes) 0.46–0.66 mm. Body pale to yellowish except frenum of scutellum white, basal gastral terga and exserted part of ovipositor sheaths brown; scape, pedicel, and legs yellowish, flagellum brownish; wings hyaline. Head about as wide as mesosoma. Antenna (Fig. 1A) with scape 2.9–3.5 times as long as wide, with cross-ridges, 2.1 times length of pedicel; F1 cylindrical, about half of pedicel length; F2 longest of funicle segments; F3 slightly shorter, F4–F6 subequal in length; longitudinal sensilla on F3 (1), F4 (1); F5 (1 or 2), and F6 (2); clava with 5 longitudinal sensilla, slightly shorter than combined length of two preceding segments. Mesosoma shorter than metasoma (Fig. 1B). Midlobe of mesoscutum with a pair of adnotaular setae. Fore wing (Fig. 1C) 8.3–8.8 times as long as wide, longest marginal seta 1.9–2.5 times maximum wing width; distal macrochaeta 1.7–1.8 times length of proximal macrochaeta; disc, besides a row of setae along each margin, with a single complete median row of setae originating behind marginal vein and a few additional, scattered setae, mostly at apex. Hind wing 26–27 times as long as wide, longest marginal seta 6.2–6.6 times maximum wing width; disc mostly bare except for admarginal rows of setae. Ovipositor notably exserted beyond apex of gaster (by

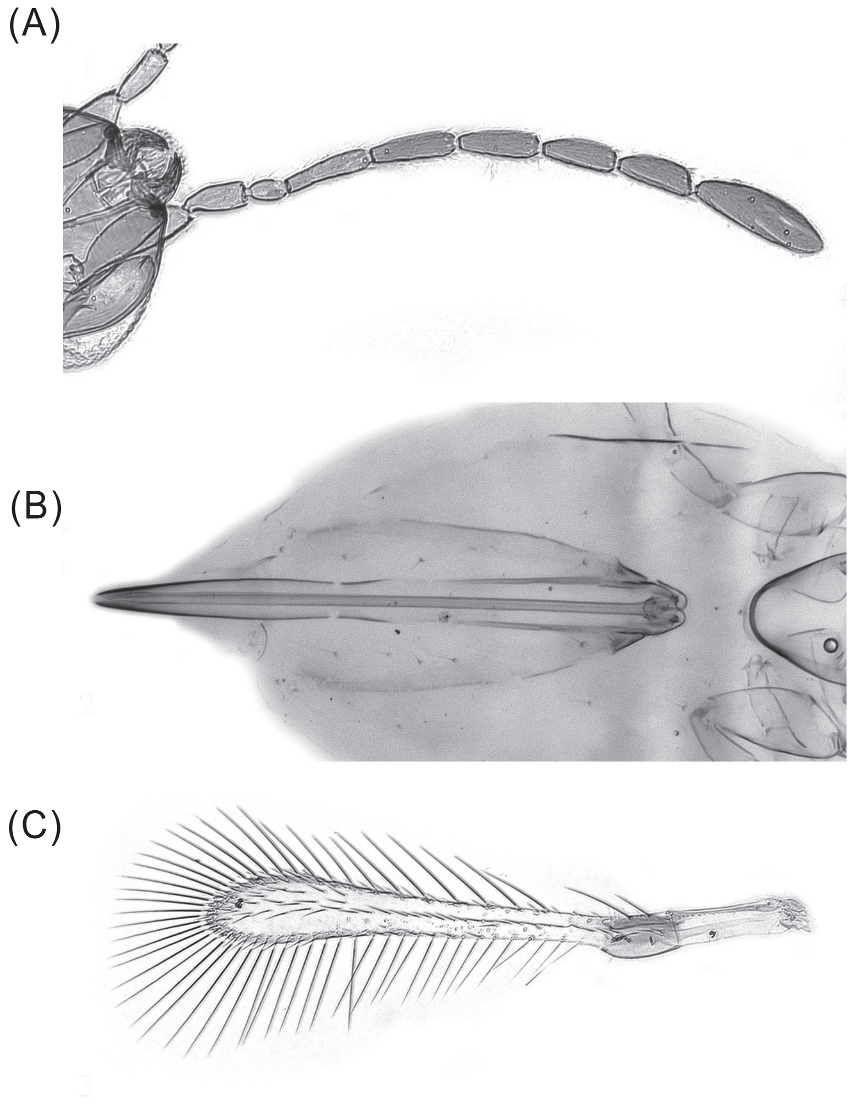

Fig. 1. *Anagrus* (*Anagrus*) *iti* (holotype female). (A) Antenna; (B) Metasoma; and (C) Fore wing.

0.15–0.16 times own length). External plates of ovipositor each with 3 distal setae. Ovipositor about 3.0 times length of protibia.

Measurements (mm) of the holotype female (as length or length : width). Body 0.805; head 0.154; mesosoma 0.258; gaster 0.406; ovipositor 0.375. A ntenna: scape 0.094; pedicel 0.045; F1 0.024; F2 0.066; F3 0.063; F4 0.058; F5 0.055; F6 0.056; clava 0.103. Fore wing 0.581: 0.07; longest marginal seta 0.133. Hind wing 0.56 : 0.021; longest marginal seta 0.13.

Male. Body length (dry-mounted paratypes) 0.43–0.56 mm. Similar to female except for the normal sexually dimorphic features such as antenna and genitalia (Fig. 2A), and the following. Body notably darker than in female, with head and most of mesonotum light brown and gaster almost entirely brown to dark brown; fore wing (Fig. 2B) about 7.5 times as

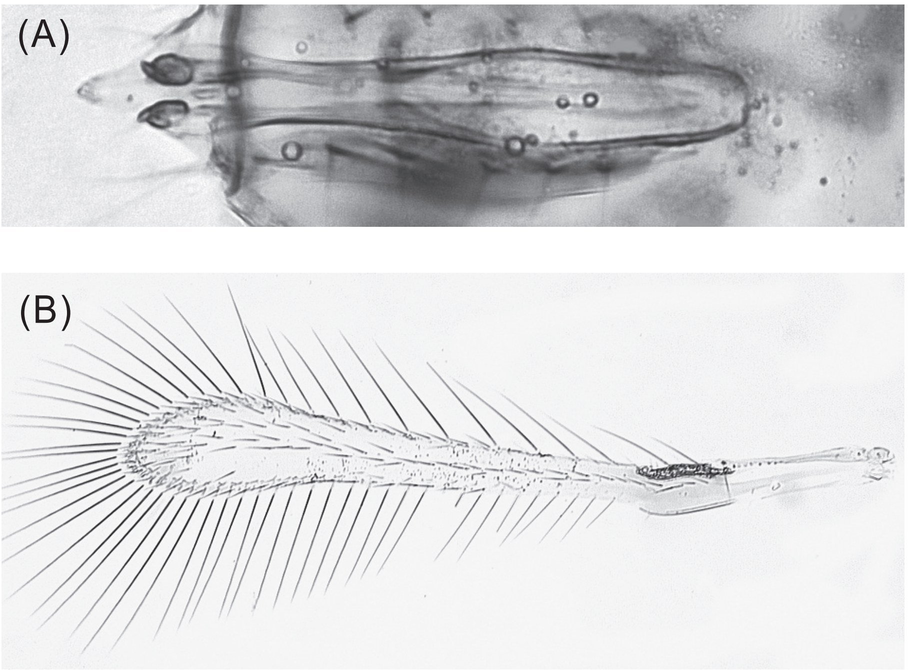

Fig. 2. *Anagrus* (*Anagrus*) *iti* (paratype male). (A) Genitalia and (B) Fore wing.

long as wide, relatively more setose than in female.

Diagnosis. The new species belongs to the *incarnatus* species group of the nominate subgenus of *Anagrus*, as defined by Chiappini *et al*. (1996). Morphologically, it is most similar to the Australasian species *A. antipodus* Triapitsyn, 2001 and also to *A. oahuensis* Triapitsyn and Beardsley, 2000 known from the Hawaiian Islands and Mexico (Triapitsyn & Beardsley 2000; Triapitsyn 2002a). *Anagrus iti* has a longitudinal sensillum on F3 of female antenna, which *A. antipodus* lacks (Triapitsyn 2001), and 3 distal setae on the external plate of the ovipositor whereas *A. oahuensis* has 2 such setae.

Etymology. The species name 'iti' means 'small' in Tahitian language, referring to the minute size of the parasitoid.

Host. The type series of this species (18 females and 5 males) was reportedly reared from one egg mass of *H. vitripennis* on *Scaevola* sp. (Goodeniaceae) containing 8 eggs (Grandgirard *et al*. 2007) [as *Anagrus* sp.], although hibiscus (*Hibiscus* sp.; Malvaceae) was indicated on the labels; it is uncertain which is the correct host plant, and they would not be confused with one another.

NOTES ON THE KNOWN EGG PARASITOIDS OF PROCONIINI IN THE WORLD

Aphelinidae

Centrodora **Förster, 1878:** Species of this genus appear to be more polyphagous egg parasitoids of some Hemiptera and are very difficult to identify. The two species reared from eggs of Proconiini remain undetermined: one *Centrodora* sp. from Tahiti and Moorea Islands, French Polynesia is a parasitoid of the non-native *H. vitripennis* (Grandgirard *et al*. 2007); the other *Centrodora* sp. was reared in Argentina from eggs of *Tapajosa rubromargin-*

ata Signoret, 1855 and *Tretogonia notatifrons* Melichar, 1926 (unpublished data).

Mymaridae

***Acmopolynema* Ogloblin, 1946:** One species of this genus, *A. sema* Schauff, was reared in Florida and Georgia, USA, from eggs of *Homalodisca insolita* (Walker 1858) (Triapitsyn, Hoddle *et al*. 2002).

***Anagrus* Haliday, 1833:** Besides *A. iti* from Tahiti described as a new species here, three other species of this genus have been recorded as egg parasitoids of Proconiini in the New World: *A. epos* Girault, 1911 in Minnesota, USA, a gregarious parasitoid of *Cuerna fenestella* Hamilton, 1970 (Hoddle & Triapitsyn 2004; Triapitsyn 2006), which was mass-reared and released without much success in California against *H. vitripennis* (Krugner *et al*. 2009; CDFA 2013), where its host specificity and other biological traits were studied by Krugner *et al*. (2008); *A. stethynioides* Triapitsyn, 2002, an occasional and poorly known parasitoid of *H. vitripennis* in Texas, USA (Triapitsyn 2006); and *A. breviphragma* Soyka, 1956 from *Dechacona missionum* (Berg 1879) in Argentina (Luft Albarracin *et al*. 2009).

***Gonatocerus* Nees ab Esenbeck, 1834:** This large and common genus contains many species that are known as egg parasitoids of various Proconiini, including those used in the biological control program against *H. vitripennis* in California (Peña *et al*. 2010; CDFA 2013), as summarized by Triapitsyn (2006) for the Nearctic region and Triapitsyn *et al*. (2010) for the Neotropical region. Details on their diversity, taxonomy, host associations and other biological traits are therefore omitted for brevity: these are readily available and too numerous to fit in this communication. All are solitary egg parasitoids, producing one wasp per host egg, except for *G. fasciatus* Girault, 1911 which is a gregarious parasitoid (Triapitsyn *et al*. 2003). Biology of several species of *Gonatocerus* was thoroughly studied (Irvin & Hoddle, 2004, 2005a, 2005b; Virla *et al*. 2005; Son *et al*. 2012). They do not appear to be too host specific but rather are usually able to attack eggs of at least several genera and species of Proconiini (Boyd & Hoddle 2007; Logarzo *et al*. 2012; Lytle, Morse *et al*. 2012). All of them belong to the subgenus *G*. (*Cosmocomoidea* Howard 1908), which also contains a large number of undescribed species from Central and South America which are likely egg parasitoids of Proconiini (Triapitsyn 2002b; Triapitsyn *et al*. 2010), thus suggesting apparent co-evolution. Triapitsyn (2012) reported the following species of *Gonatocerus* from eggs of *H. vitripennis* in California: *G. ashmeadi* Girault, 1915, *G. fasciatus* (intentionally introduced), *G. incomptus* Huber, 1988, *G. morgani* Triapitsyn, 2006, *G. morrilli* (Howard 1908) (intentionally introduced), *G. novifasciatus* Girault, 1911, *G. triguttatus* Girault, 1916 (intentionally introduced), and *G. walkerjonesi* Triapitsyn, 2006. The southern and southeastern USA strains of *G. ashmeadi* were released in California against *H. vitripennis* (Morgan *et al*. 2002; Pilkington *et al*. 2005). *Gonatocerus ashmeadi* was found self-introduced in Oahu Island, Hawaii (USA), where it provides a good control of *H. vitripennis*; it was intentionally and successfully introduced into French Polynesia for very effective biological control against *H. vitripennis* (Grandgirard *et al*. 2008a, 2008b). It was also found self-introduced in Easter Island, Chile (Triapitsyn 2006). Paradell *et al*. (2012) summarized the known egg parasitoids of Proconiini in Argentina, the majority of which are *Gonatocerus* species described or revised taxonomically by Triapitsyn *et al*. (2006, 2007, 2008, 2010); *T. rubromarginata* is the most thoroughly studied host of many of these parasitoids.

Palaeoneura **Waterhouse, 1915:** One undetermined species of this genus was reared in Tahiti Island from eggs of *H. vitripennis* (Grandgirard *et al*. 2007).

Polynema **Haliday, 1833:** An undescribed species of *P.* (*Doriclytus*) sp. was reared in California, USA from eggs of *H. vitripennis*; it is only an occasional parasitoid of this host (Triapitsyn 2012). One or two other *P.* (*Doriclytus*) spp. were reared in Argentina from sentinel eggs of *T. rubromarginata* (G. A. Logarzo and S. V. Triapitsyn, unpublished).

Trichogrammatidae

Members of several genera are gregarious egg parasitoids of various Proconiini, all in the New World (Triapitsyn 2003); trichogrammatids are generally relatively more common on grassy vegetation and in dry habitats.

Burksiella **De Santis, 1957:** *Burksiella spirita* (Girault 1918) is a common egg parasitoid of *H. vitripennis* and also of *Oncometopia orbona* (Fabricius 1798) in the southeastern USA (Triapitsyn 2003 [as *Zagella spirita* (Girault)], 2012); a related form, which may or may not belong to this species, was reared in Montana, USA, from eggs of *Cuerna sayi* Nielson, 1965 (Triapitsyn *et al*. 2011). One or two different, undetermined species *Burksiella* sp.(p) was/were reported from eggs of *Homalodisca liturata* Ball, 1901 in Mexico (Triapitsyn & Bernal 2009). *Burksiella platensis* (De Santis 1957) is known from eggs of *T. rubromarginata* in Argentina (Triapitsyn 2003).

Ittys **Girault, 1911:** One undescribed species of this genus was reported from eggs of *H. liturata* in Mexico (Triapitsyn & Bernal 2009).

Oligosita **Walker, 1851:** An *Oligosita* sp., possibly *O. americana* Girault, 1909, was reared in Georgia from eggs of *H. insolita* (Turner & Pollard 1959; Triapitsyn 2003). Undetermined *Oligosita* spp. were also reared from eggs of two species of Proconiini in Argentina (Paradell *et al*. 2012); at least one of these is, however, a member of *Pseudoligosita* Girault, 1913 (Triapitsyn & Bernal 2009).

Paracentrobia **Howard, 1897:** *Paracentrobia acuminata* (Ashmead 1888) was reared in Florida and Georgia from eggs of *Cuerna costalis* (Fabricius 1803), *H. insolita*, and *H. vitripennis* (Turner and Pollard 1959; Triapitsyn 2003 [as *Paracentrobia* (*Brachistella*) sp.]; Tipping *et al*. 2005). Also *P. americana* (Girault 1917) is a parasitoid of *H. insolita* in Florida (Tipping *et al*. 2005). In Argentina, *P. tapajosae* Viggiani, 2009 is known from eggs of *T. rubromarginata* when Virla *et al*. (2009) studied biology of the parasitoids. Undetermined *Paracentrobia* spp. were also reared from eggs of three other species of Proconiini in Argentina (Paradell *et al*. 2012).

Pseudoligosita**:** Two species of this genus were reared from eggs of Proconiini: *P. plebeia* (Perkins 1912) from *H. liturata* in Mexico [Triapitsyn & Bernal 2009 (as *Pseudoligosita* sp.); Triapitsyn 2012] and also from eggs of *T. rubromarginata* in Argentina (Triapitsyn & Bernal 2009); and an undetermined and apparently undescribed *Pseudoligosita* sp. from eggs of *H. vitripennis* in California (Triapitsyn 2012). Biological traits of *P. plebeia*, which was reared under quarantine conditions in California on eggs of *H. vitripennis*, were reported by Triapitsyn & Bernal (2009) and Lytle, Bernal *et al*. (2012).

Ufens **Girault, 1911:** *Ufens ceratus* Owen, 2005 and *U. principalis* Owen, 2005 are common egg parasitoids of *H. liturata* and *H. vitripennis* in Mexico and USA (Al-Wahaibi *et al*. 2005; Triapitsyn & Bernal 2009; Triapitsyn 2012), and *U. ceratus* is also known from *Oncometopia clarior* (Walker 1851) in Mexico [Triapitsyn, Bezark *et al*. 2002 (as *Ufens* sp.)]. *Ufens niger* (Ashmead 1888) was reported from eggs of *C. costalis* and *Homalo-*

disca sp. (Turner & Pollard 1959; Triapitsyn 2003).

***Zagella* Girault, 1918:** The sharpshooter *T. rubromarginata* is a known host of *Z. delicata* De Santis, 1970, its biology was reported by Logarzo *et al*. (2004). Undetermined *Zagella* spp. were also reared from eggs of three other species of Proconiini in Argentina (Paradell *et al*. 2012).

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法屬大溪地島之 *Anagrus* 屬一新種 (膜翅目：纓小蜂科) 與全球之脊大葉蟬族 (半翅目：葉蟬科：脊大葉蟬族) 卵寄生蜂記述

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摘要

Triapitsyn1, S. V。2013。法屬大溪地島之 *Anagrus* 屬一新種 (膜翅目：纓小蜂科) 與全球之脊大葉蟬族 (半翅目：葉蟬科：脊大葉蟬族) 卵寄生蜂記述。台灣農業研究 62(4):289–299。

褐透翅尖頭大葉蟬 [*Homalodisca vitripennis* (Germar)] 原產於美國東南至墨西哥東北區域，目前已成為法屬波里尼西亞大溪地島的入侵物種。本研究描述採自大溪地島本種葉蟬蟲卵之纓小蜂科一個新種 – 微纓小蜂 [*Anagrus* (*Anagrus*) *iti* sp. n.]，並針對新世界區的脊大葉蟬族之卵寄生蜂 (包括膜翅目的蚜小蜂科、纓小蜂科及赤眼卵蜂科)，提供分類學與生物學的研究回顧。這部份又以傳播植物病原細菌 *Xylella fastidiosa* 最具效力的媒介昆蟲 - 褐透翅尖頭大葉蟬為重點，包括此種葉蟬在非原產地的卵寄生蜂與生物防治之相關研究。

關鍵詞：葉蟬、Proconiini、卵寄生蜂、*Anagrus*、生物防治。

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A List of Aspidiotinae (Hemiptera: Coccoidea: Diaspididae) Deposited at the Insect Collection of Taiwan Agricultural Research Institute

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Abstract

Chen, S. P., J. Y. Wong, and W. J. Wu. 2013. A list of Aspidiotinae (Hemiptera: Coccoidea: Diaspididae) Deposited at the Insect Collection of Taiwan Agricultural Research Institute. J. Taiwan Agric. Res. 62(4):300–320.

A list of 42 species in 23 genera of Aspidiotinae (Hemiptera: Sternorrhyncha: Coccoidea: Diaspididae) with 391 slide mounted specimens deposited at Insect Collection of the Taiwan Agricultural Research Institute (TARI) is provided. The specimens were mainly collected during Japanese Colonial Period. Synonyms, host plants, distribution and photographs of slide-mounted specimen are also provided for each species.

Key words: Aspidiotinae (Diaspididae), Slide-mounted specimens, Taiwan Agricultural Research Institute (TARI).

INTRODUCTION

Scale insects (Hemiptera: Sternorrhyncha: Coccoidea) of Taiwan have been studied extensively by serveral western and Japanese entomologists such as Green, Ferris and Takahashi since 1923 (Tao 1978). Most of the slide-mounted specimens of Aspidiotinae (Hemiptera: Sternorrhyncha: Coccoidea: Diaspididae) collected by Takahashi were deposited at the Insect Collection, Division of Applied Zoology, Taiwan Agricultural Research Institute (TARI). Among them, a great number of slide-mounted specimens were classified by Takahashi. However, a considerable number of species names used by Takahashi have been changed or synonymized with other names. In this study, taxonomic status of all slide-mounted specimens were revised based on the modern taxonomic studies.

MATERIALS AND METHODS

A total of 391 slide-mounted specimens were examined by a Leitz DMRB light microscope (Leica, Milton Keynes, U.K.) in this study. Distribution data of each species were compiled based on the information provided on the slide-mounted specimens deposited at the TARI Insect Collection. Photographs of slide-mounted specimens were taken for each species. According to Chu & Yamanaka (1973, 1974, 1975) and the Taiwan Geographic Name Information Systems (http://placesearch.moi.

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gov.tw/search/index.php), the old name and current name of collection locality records were included in the paper.

RESULTS

A total of 42 species (23 genera) of Aspidiotinae were identified. Among them, there were 15 species described by Takahashi, 2 species described by Kuwana. The following list provides comprehensive information on the nomenclature, synonymies, host plants, and distribution of each species. Table 1 is a check list of the old name and current name of collection locality records included in the paper (Chu & Yamanaka 1973, 1974, 1975).

***Abgrallaspis cyanophylli* (Signoret 1869)** (Fig. 1A) (Tao 1989; Wong *et al*. 1999)

Original spelling on label: *Aspidiotus cyanophylli* Signt. (8 slides)

Distribution: Taiwan (Heito: Heito, Kuraru;

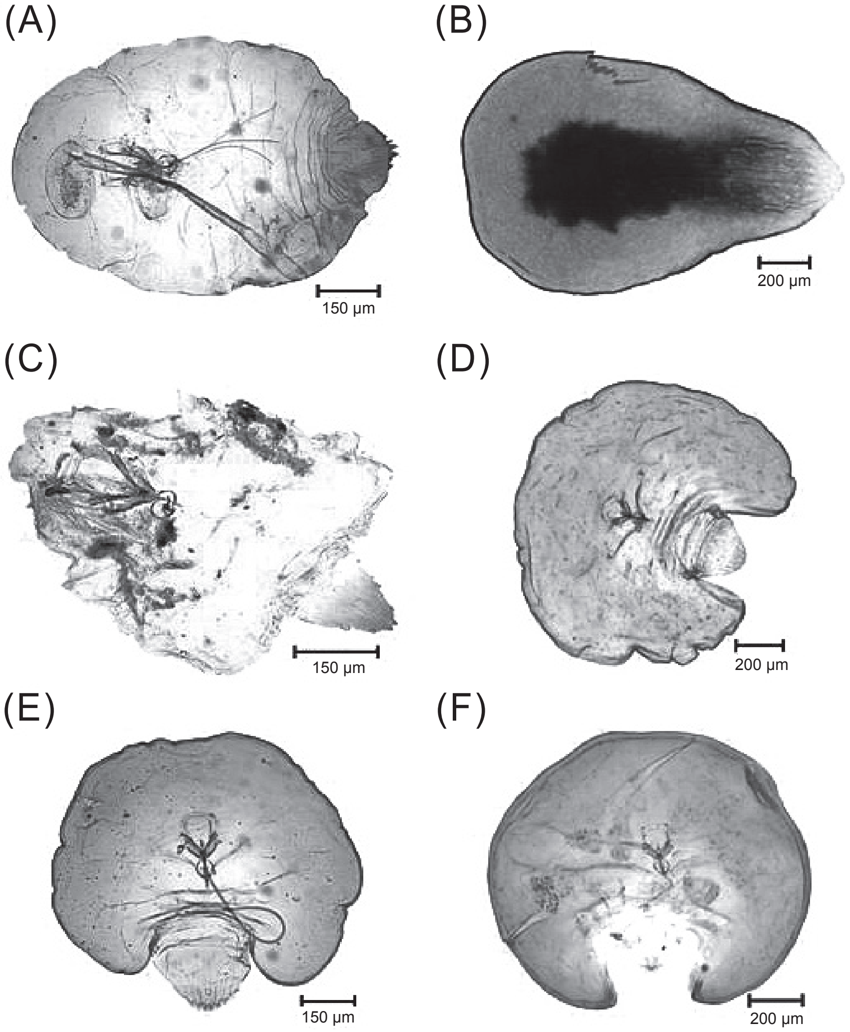

Fig. 1. (A) *Abgrallaspis cyanophylli* (Signoret 1869); (B) *Aonidia formosana* (Takahashi 1935); (C) *Aonidia rarasana* (Takahashi 1934); (D) *Aonidiella aurantii* (Maskell 1879); (E) *Aonidiella citrina* (Coquillett 1891); and (F) *Aonidiella godfreyi* (Takahashi 1942).

Table 1. A check list of the old name, current name and Chinese name of collection localities included in the paper.

Old name	Current name	Chinese name
Bokoto	Penghu	澎湖
Botel Tobago	Taitung: Lanyu	台東：蘭嶼
Buruburu	Taitung: Haiduan	台東：海端
Chipponsan	Taitung: Zhiben Mountain	台東：知本山
Chushinron near Rokki	Kaohsiung: Paoshan nera Liouguei	高雄：寶山 近 六龜
Daichikko	Taitung: Dazhu	台東：大竹
Garambi, Garanbi	Pingtung: Eluanbi (Ouluanpi)	屏東：鵝鑾鼻
Gyochi	Nantou: Yuchi	南投：魚池
Habon	Nantou: Holuan	南投：合巒
Habon near Musha	Nantou: Holuan near Wushe	南投：合巒 近 霧社
Heitin	Taoyuan: Pinglin	桃園：坪林
Heito	Pingtung	屏東
Hinokiyama	New Taipei City: Kuaishan	新北市：檜山
Hori, Horin	Nantou: Puli	南投：埔里
Hsintien	New Taipei City: Xindian	新北市：新店
Ikenohata	Yilan: Chihtuan	宜蘭：池端
Iran	Yilan	宜蘭
Kagi	Chiayi	嘉義
Kahodai	Taichung: Jiabaotai (Chiapaotai)	台中：佳保台
Kakayo, Kakayom	Taitung: Jiadai (Chiachiadai)	台東：嘉代 (嘉嘉代)
Kanaron	Taitung: Jinlun (Chinlun)	台東：金崙
Karenko	Hualien	花蓮
Kashoto	Taitung: Ludao (Lyudao)	台東：綠島
Kiirun	Keelung	基隆
Kinyan	Yilan: Jinyang (Chinyang)	宜蘭：金洋
Komoko	Hsinchu: Xinfeng (Sinfong)	新竹：新豐
Kotosho	Taitung: Lanyu	台東：蘭嶼
Kowarun	Taitung: Daren	台東：達仁
Kuraru	Pingtung: Sheding	屏東：社頂
Mako	Penghu: Magong	澎湖：馬公
Maruyama	Taipei: Yuanshan	台北：圓山
Mato	Tainan: Madou	台南：麻豆
Murorafu, Muroruafu, Mururoafu	Yilan: Jialuo Mountain (Chialoshan)	宜蘭：加羅山
Mutosan	Pingtung: Wutoushan	屏東：霧頭山
Nisui	Changhua: Ershui	彰化：二水
Onoe near Musha	Nantou: Weishang near Wushe	南投：尾上 近 霧社
Raisha	Pingtung: Laiyi	屏東：來義
Rarasan	New Taipei City: Lala Mountain (Lalashan)	新北市：拉拉山
Rirei	Taichung: Lileng	台中：裡冷
Riyohen	Yilan, Liuxingshe	宜蘭 , 流興社

Table 1. A check list of the old name, current name and Chinese name of collection localities included in the paper. (continued)

Old name	Current name	Chinese name
Ropi near Heito	Pingtung	屏東：近墾丁
Shijukei	Pingtung: Sichongxi (Suchungchi)	屏東：四重溪
Shimpo, Shimpro	Hsinchu: Xinpu	新竹：新埔
Shinten	New Taipei City: Xindian	新北市：新店
Shintiku	Hsinchu	新竹
Shin-Taiheizan	Yilan: Taiping Mountain (Taipingshan)	宜蘭：太平山
Shirin	Taipei: Shilin	台北：士林
Shoka	Changhua	彰化
Shokwa	Tainan, Xinhua, Damujiang	台南：新化：大目降
Sozan	Taipei: Yangmingshan	台北：陽明山
Suisha	Nantou: Shuishe	南投：魚池：水社
Suo	Yilan: Su'ao	宜蘭：蘇澳
Taichu	Taichung	台中
Taiheizan, Taihezan, Taiheisan	Yilan: Taiping Mountain (Taipingshan)	宜蘭：太平山
Taihoku, Taipei	Taipei	台北
Tainan	Tainan	台南
Taito, Taitocho	Taitung	台東
Taka, Takaoshu	Kaohsiung	高雄
Tamsu, Tansui	New Taipei City: Danshui (Danshuei)	新北市：淡水
Toran	Taitung: Dulan (Doulan)	台東：都蘭
Urai	New Taipei City: Wulai	新北市：烏來
Washoshu	Nantou: Hoshe (Washa)	南投：和社
Yensuiko	Tainan: Yanshui	台南：鹽水

Nantou: Suisha; Taihoku: Shinten, Taihoku).

Host plant: *Camellia japonica*, *Gardenia* sp., *Mangifera indica*, *Palm* sp., *Tectona grandis*.

Syn.: *Aspidiotus cyanophylli* Signoret, 1869; *Aspidiotus* (*Aspidiotus*) *cyanophylli* (Signoret 1869); *Aspidiotus* (*EvAspidiotus*) *cyanophylli* (Signoret 1869); *Furcaspis cyanophylli* (Signoret 1869); *Furcaspis* (*Aspidiotus*) *cyanophylli* (Signoret 1869); *Hemiberlesia cyanophylli* (Signoret 1869); *DiAspidiotus cyanophylli* (Signoret 1869); *Aspidiotus* (*Hemiberlesia*) *cyanophylli* (Signoret 1869).

Aonidia formosana **Takahashi, 1935** (Fig. 1B)

(Takahashi 1935; Borchsenius 1966; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Aonidia tentaculata formosana* Tak. (2 slides)

Distribution: Taiwan (Nantou: Habon, Habon near Musha).

Host plant: *Cinnamomum* sp.

Syn.: *Aonidia tentaculata formosana* Takahashi, 1935.

Note: Syntypes, female [collected from Taiwan: Habon near Musha, on *Cinnamomum* sp., on 10-VIII-1934.by R. Takahashi (#15-2-5-1–2)].

Aonidia rarasana **Takahashi, 1934** (Fig. 1C)

(Takahashi 1934; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Aonidia rarasana* Takah. (3 slides)
Distribution: Taiwan (Taipei: Rarasan).
Syn.: *Gymnaspis rarasana* (Takahashi, 1934).
Note: Syntypes, female [collected from Taiwan: Rarasan, near Urai, on undetermined host, on 31-VII-1933, by R. Takahashi (#15-2-4-1–3)].

***Aonidiella aurantii* (Maskell 1879)** (Fig. 1D)
(Maskell 1879; Berlese 1895;Tao 1989; Wong et al. 1999)

(1) Original spelling on label: *Aspidiotus aurantii* Mask. (7 slides)
Distribution: China (Foochow); Taiwan (Heito: Heito, Shijukei; Taihoku: Tansui; Taito); USA (Saipan: Garapan).

(2) Original spelling on label: *Aonidiella aurantii* Mask. (21 slides)
Distribution: India (Bannu; Darband; Goriwala: Shah Pasand; Lyallpur); Japan (Kyushu: Kagoshima); Micronesia (Caroline Isls.: Truk: Toloas-Erin); Palau Isls. (Babeldaob); Taiwan (Giran: Muroruafu, Mururoafu; Heito: Garambi, Kuraru; Taihoku: Shirin, Taihoku); Thailand (Ubon).

(3) Original spelling on label: *Chrysomphalus aurantii* Mask. (26 slides)
Distribution: Japan (Yamaguchi); Palau Isls. (Korror); Taiwan (Giran: Ikenohata, Murorafu, Riyohen; Heito; Kagi; Naii; Taichu: Kahodai; Taihoku; Tainan: Mato; Taito: Daichikko, Kakayo; Takao: Chushinron near Rokki, Takao).
Host plant: *Ardisia* sp., *Asparagus* sp., *Bischofia* sp., *Citrus Grundis*, *Citrus* sp., *Citrus tachibana*, *Distylium racemosum*, *Gelonium aequoreum*, *Illicium tashiroi*, *Mangifera indica*, Malta fruit, *Padocarpus* sp., *Pandanus* sp., *Pinus* sp., *Scaevola taccada*, *Scolopia oldhami*, *Yucca gloriosa*.
Syn.: *Aspidiotus aurantii* Maskell, 1879; *Aonidia gennadii* Targioni Tozzetti, 1881; *Aspidiotus coccineus* Gennadius, 1881; *Aspidiotus citri* Comstock, 1881; *Aspidiotus* (*Aonidiella*) *aurantii* (Maskell, 1879); *Chrysomphalus* (*Aonidiella*) *aurantii* (Maskell, 1879); *Aspidiotus* (*Chrysomphalus*) *aurantii* (Maskell, 1879); *Chrysomphalus citri* (Comstock, 1881); *Aonidiella coccineus* (Gennadius, 1881); *Aonidiella gennadi* (Targioni Tozzetti, 1881); *Chrysomphalus coccineus* (Gennadius, 1881).

***Aonidiella citrina* (Coquillett 1891)** (Fig. 1E)
(Lindinger 1914; Tao 1989)

(1) Original spelling on label: *Aonidiella citrina* Coq. (7 slides)
Distribution: India (Bannu); Japan (Kyushu: Kagoshima, Oita); Taiwan (Pintung: Garanbi).

(2) Original spelling on label: *Chrysomphalus citrina* Coq. (2 slides)
Distribution: Taiwan (Yilan: Riyohen).
Host plant: *Citrus* sp., *Gelonium aequoreum.*, *Syzygium cumini*.
Syn.: *Aspidiotus citrinus* Coquillett, 1891; *Aspidiotus aurantii citrinus* (Coquillett 1891); *Aspidiotus* (*Aonidiella*) *aurantii citrinus* (Coquillett 1891); *Aonidiella aurantii citrina* (Coquillett 1891); *Chrysomphalus aurantii citrinus* (Coquillett 1891); *Chrysomphalus citri*nus (Coquillett 1891); *Aonidiella citrina* (Coquillett 1891); *Chrysomphalus citrina* (Coquillett 1891).

***Aonidiella godfreyi* Takahashi, 1942** (Fig. 1F)
(Takahashi 1942; Ben-Dov & Miller 2010)

Original spelling on label: *Aonidiella godfreyi* Takah. (1 slide)
Distribution: Thailand (Chiengmai).
Host plant: undetermined tree.
Note: Syntype, female [collected from Thailand: Chiengmai, on an undetermined tree , 02-IV-1940, by R. Takahashi (#16-1-3-1)].

***Aonidiella inornata* McKenzie, 1938** (Fig. 2A)
(McKenzie 1938; Tao 1989)
Original spelling on label: *Aonidiella inornata* McKenzie. (11 slides)
Distribution: Japan (Kyushu: Kagoshima); Micronesia (Caroline Isls.: Oleai-Atoll: Falulap; Yap: Okao); Palau Isls. (Korror: Arabaketsu, Korror, Makalai); Taiwan (Tainan).
Host plant: *Campno sperma*, *Carica papaya*, *Cocos nucifera*, *Mascarema lagenicaulis*, *Piper kadsura*, *Plumeria acuminata*.

***Aonidiella orientalis* (Newstead 1894)** (Fig. 2B) (Newstead 1894; McKenzie 1938; Chou 1985)
Original spelling on label: *Aonidiella orientalis* Newst. (3 slides)
Distribution: India (Lyallpur); Malaysia (Malaya: Serdang).
Host plant: Cocos nucifera, Dalbergia sp..
Syn.: *Aspidiotus orientalis* Newstead, 1894; *Aspidiotus osbeckiae* Green, 1896; *Aspidiotus* (*DiAspidiotus*) *osbeckiae* (Newstead, 1894); *Aspidiotus* (*EvAspidiotus*) *orientalis* (Newstead, 1894); *Aspidiotus* (*Chrysomphalus*) *pedronis* Green, 1905; *Aspidiotus* (*Aonidiella*) *taprobanus* Green, 1905; *Chrysomphalus pedronis* (Newstead, 1894); *Chrysomphalus taprobanus* (Newstead, 1894); *Aspidiotus* (*Aonidiella*) *cocotiphagus* Marlatt, 1908; *Aspidiotus cocotiphagus* (Newstead, 1894); *Chrysomphalus orientalis* (Newstead, 1894); *Chrysomphalus pedroniformis* Cockerell & Robinson, 1915; *Furcaspis cocotiphaga* (Newstead, 1894); *Furcaspis orientalis* (Newstead, 1894); *Aonidiella taprobana* (Newstead, 1894); *Aspidiotus orientalis cocotiphagus* (Newstead, 1894); *Aspidiotus* (*Furcaspis*) *orientalis* (Newstead, 1894); *Aonidiella orientalis* (Newstead, 1894); *Aonidiella cocotiphagus* (Newstead, 1894); *Aonidiella pedroniformis* (Newstead, 1894); *Aonidiella pedronis* (Newstead, 1894).

***Aonidiella sotetsu* (Takahashi 1933)** (Fig. 2C)
(Takahashi 1933; McKenzie 1938; Ben-Dov & Miller 2010)
Original spelling on label: *Chrysomphalus sotetsu* Takahashi. (4 slides)
Distribution: Japan (Loochoo: Ishigaki, Miyako).
Host plant: *Cycas* sp., *Ficus retusa*.
Syn.: *Chrysomphalus sotetsu* Takahashi, 1933.
Note: Syntypes, female [collected from Japan: Ishigaki, Miyako, on *Cycas revoluta* and *Ficus retusa*. III-1932 and VII-1932 by S. Yasukawa (#19-2-1-1–4)].

***Aonidiella taxus* Leonardi, 1906** (Fig. 2D)
(Leonardi 1906; Sanders 1909; McKenzie 1938; Tao 1989)
(1) **Original spelling on label:** *Aonidiella taxus* Leon. (2 slides)
Distribution: Japan (Kyushu: Kagoshima, Tanegashima).
(2) **Original spelling on label:** *Chrysomphalus taxus* Leon. (2 slides)
Distribution: Taiwan (Takao).
Host plant: *Ardisia sieboldii*, *Nageia nagi*, *Podocarpus* sp..
Syn.: *Aonidiella taxa* (Leonardi, 1906); *Chrysomphalus taxus* (Leonardi, 1906); *Aspidiotus taxus* (Leonardi, 1906).

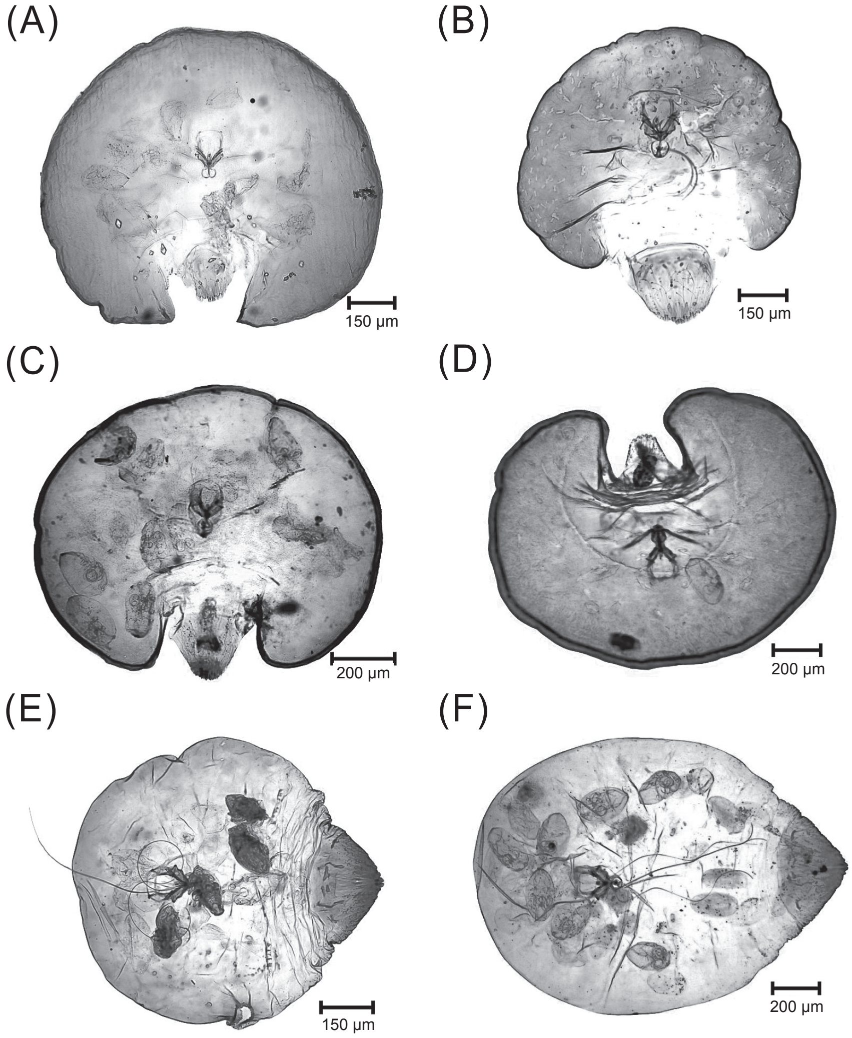

Fig. 2. (A) *Aonidiella inornata* (McKenzie 1938); (B) *Aonidiella orientalis* (Newstead 1894); (C) *Aonidiella sotetsu* (Takahashi 1933); (D) *Aonidiella taxus* (Leonardi 1906); (E) *Aspidiella phragmitis* (Takahashi 1931); and (F) *Aspidiella sacchari* (Cockerell 1893).

***Aspidiella phragmitis* (Takahashi 1931)** (Fig. 2E) (Takahashi 1931c; Ferris 1941; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Aspidiotus phragmitis* Tak. (10 slides)

Distribution: Taiwan (Giran: Kinyan, Suo; Taihoku: Shinten, Taihoku; Taito: Daichikko).

Host plant: *Phragmites* sp..

Syn.: *Aspidiotus phragmitis* Takahashi, 1931; *Aspidiotus miscanthii* Kuwana, 1931; *Chortinaspis phragmitis* (Takahashi 1931).

Note: Syntypes, female [collected from Taiwan: Suo, Daichikko near Daibu, on *Phragmites* sp. , 20–25-XI-1930 by R. Takahashi (#17-4-4-1–4)].

***Aspidiella sacchari* (Cockerell 1893)** (Fig. 2F) (Cockerell 1893a; MacGillivray 1921; Chou 1985)

Original spelling on label: *Aspidiotus sac-*

chari Ckll. (3 slides)

Distribution: Japan (Okinawa: Daito-jima); USA (Saipan: Garapan)

Host plant: *Phragmites* sp..

Syn.: *Aspidiotus sacchari* Cockerell, 1893; *Aspidiotus* (*Aspidiotus*) *sacchari* (Cockerell 1893); *Aspidiotus* (*Aspidiella*) *sacchari* (Cockerell 1893); *Targionia sacchari* (Cockerell 1893); *Targionia* (*Aspidiella*) *sacchari* (Cockerell 1893).

***Aspidiotus destructor* Signoret, 1869** (Fig. 3A) (Tao 1989; Wong *et al*. 1999)

(1) **Original spelling on label:** *Aspidiotus destructor* Signt. (34 slides)

Distribution: Cambodia (Angkor); China (Shanghai); Japan (Kyoto); Malaysia (Malaya: K. Lumpur); Palau Isls. (Angaur: Saipan-Kitamura; Babeldao: Ngiwal-Ngarard; Korror); Taiwan (Giran: Ikenohata; Taihoku: Hinokiyama, Shirin, Taihoku; Tainan); Thailand (Chiengmai; Mt. Sutep); USA (Rota: Tatacho-Sonson; Saipan: Garapan, Saipan).

(2) **Original spelling on label:** *Aspidiotus transparens* Green. (10 slides)

Distribution: China (Taichow: Checkiang); Malaysia (Malaya: K. Lumpur); Taiwan (Heito: Garanbi, Mutosan; Taito: Kotosho, Toran; Takao).

Host plant: *Aucuba japonica*, *Avocado* sp., *Carica papaya*, *Citrus* sp., *Cleyera japonica*, *Dimocarpus longan*, *Dypsis lutescens*, *Ficus elastic*, *Ficus retusa*, *Ficus* sp., *Illicium anisatum*, *Lithocarpus* sp., *Mangifera indica*, *Musa* sp., *Palm*, *Panaanus* sp., *Plumeria rubra*, *Psychotrya* sp., *Scolopia* sp., *Stephania cepharantha*, *Trachycarpus wagnerianus*, *Vitis* sp..

Syn.: *Aspidiotus cocotis* Newstead, 1893; *Aspidiotus fallax* Cockerell, 1893; *Aspidiotus vastatrix* Leroy, 1896; *Aspidiotus destructor fallax* (Signoret 1869); *Aspidiotus* (*Aspidiotus*) *transparens* (Signoret 1869); *Aspidiotus* (*Aspidiotus*) *destructor* (Signoret 1869); *Aspidiotus transparens* Green, 1890; *Aspidiotus transparens simillimus* Cockerell, 1898.

***Aspidiotus excisus* Green, 1896** (Fig. 3B) (Green 1896b; MacGillivray 1921; Williams & Watson 1988; Tao 1989; Wong *et al*. 1999)

Original spelling on label: *Aspidiotus excisus* Green. (18 slides)

Distribution: Palau Isls. (Korror); Taiwan (Heito: Garanbi, Raisha; Nantou: Hori, Suisha; Taihoku: Shinten, Sozan, Taihoku, Washoshu; Taito: Buruburu, Kashoto, Kowarun; Urai).

Host plant: *Clerodendrum inerme*, *Elephantopus* sp., *Heliotropium foertherianum*, *Hoya* sp., *Glochidion* sp., *Rhododendron* sp., *Rhus* sp., *Viburnum* sp..

Syn.: *Aspidiotus* (*Aspidiotus*) *excisus* (Green 1896); *Aspidiotus* (*EvAspidiotus*) *excisus* (Green 1896); *TemnAspidiotus excisus* (Green 1896).

***Chrysomphalus aonidum* (Linnaeus 1758)** (Fig. 3C) (Linnaeus 1758; Cockerell 1899b; Tao 1989)

Original spelling on label: *Chrysomphalus aonidum* L. (24 slides)

Distribution: China (Hainan); Japan (Kyushu: Tanegashima); Micronesia (Caroline Isls.: Ponape: Kolonia); Taiwan (Giran: Giran, Taihezan; Heito: Garanbi, Heito; Kagi; Kiirun; Nantou: Hori; Shoka: Nisui; Taihoku; Tainan: Yensuiko; Taito; Toen: Heitin).

Host plant: *Areca catechu*, *Citrus* sp., *Cycas* sp., *Galium echinocarpum*, *Gentiana*

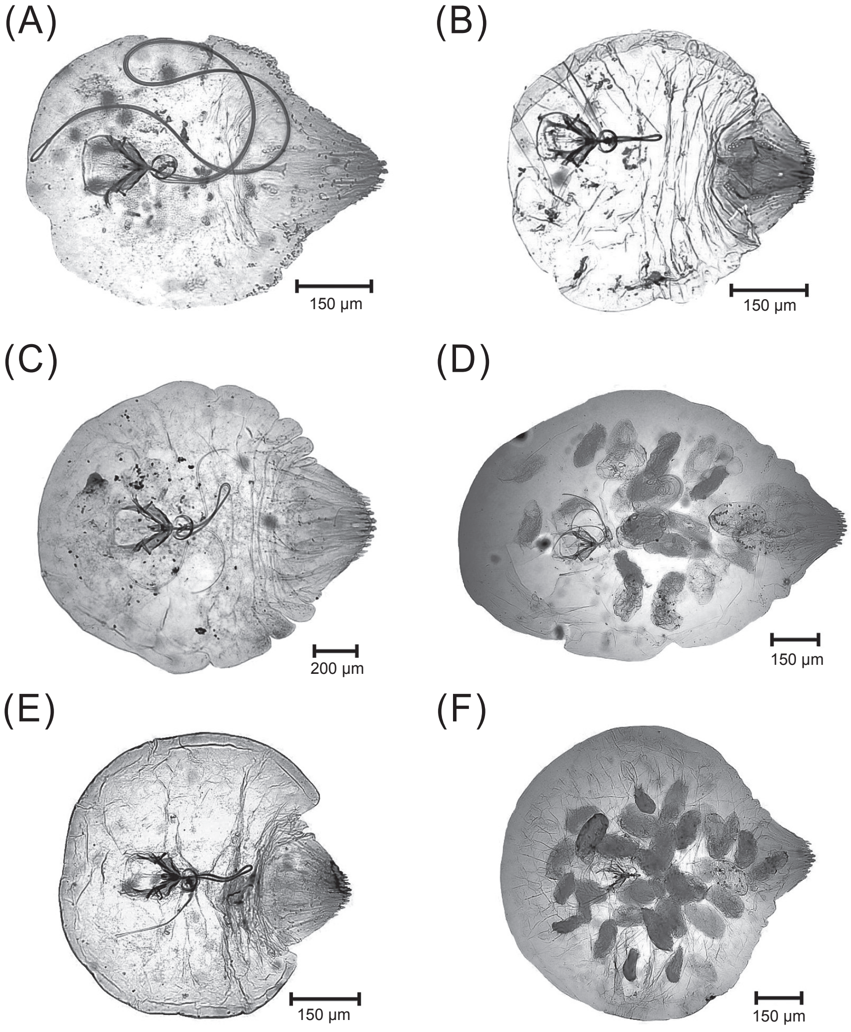

Fig. 3. (A) *Aspidiotus destructor* (Signoret 1869); (B) *Aspidiotus excisus* (Green 1896); (C) *Chrysomphalus aonidum* (Linnaeus 1758); (D) *Chrysomphalus bifasciculatus* (Ferris 1938); (E) *Chrysomphalus dictyospermi* (Morgan 1889); and (F) *Chrysomphalus pinnulifer* (Maskell 1891).

scabra, *Kandelia obovata*, *Mahonia japonica*, Palm, P*andanus odoratissimus*, *Pinus* sp., *Podocarpus macrophylla*, *Rosa* sp., *Thea* sp..

Syn.: *Coccus aonidum* Linnaeus, 1758; *Chrysomphalus ficus* Ashmead, 1880; *Aspidiotus* (*Chrysomphalus*) *ficus* (Ashmead 1880); *Aspidiotus* (*Chrysomphalus*) *aonidum* (Linnaeus 1758); *Aspidiotus aonidum* (Linnaeus 1758).

***Chrysomphalus bifasciculatus* Ferris, 1938**
(Fig. 3D) (Tao 1989)

Original spelling on label: *Chrysomphalus bifasciculatus* Ferris. (4 slides)

Distribution: Taiwan (Heito: Garanbi; Nantou: Suisha).

Host plant: *Cycas* sp., *Pandanus* sp..

***Chrysomphalus dictyospermi* (Morgan 1889)**
(Fig. 3E) (Morgan 1889; Maskell 1895; Tao 1989)

Original spelling on label: *Chrysomphalus*

dictyospermi Morg. (9 slides)

Distribution: Palau (Korror); Samoa; Taiwan (Heito; Shintiku: Komoko, Shimpro; Taihoku).

Host plant: *Cocos nucifera*, *Kandelia obovata*, *Mangifera indica*, *Phragmites* sp., *Pinus* sp..

Syn.: *Aspidiotus dictyospermi* Morgan, 1889; *Aspidiotus dictyospermi arecae* Newstead, 1893; *Aspidiotus mangiferae* Cockerell, 1893; *Aspidiotus arecae* (Newstead 1893); *Aspidiotus dictyospermi jamaicensis* Cockerell, 1894; *Chrysomphalus dictyospermi* (Morgan 1889); *Chrysomphalus minor* Berlese in Berlese & Leonardi, 1896; *Aspidiotus minor* (Berlese in Berlese & Leonardi 1896); *Aspidiotus* (*Chrysomphalus*) *dictyospermi* (Morgan 1889); *Aspidiotus* (*Chrysomphalus*) *dictyospermi arecae* (Morgan 1889); *Aspidiotus* (*Chrysomphalus*) *dictyospermi jamaicensis* (Cockerell 1894); *Aspidiotus* (*Chrysomphalus*) *mangiferae* (Morgan 1889); *Aspidiotus* (*Chrysomphalus*) *minor* (Berlese in Berlese & Leonardi 1896); *Chrysomphalus mangiferae* (Cockerell 1893); *Chrysomphalus dictyospermi arecae* (Morgan 1889); *Chrysomphalus dictyospermi mangiferae* (Morgan 1889); *Chrysomphalus dictyospermi minor* (Morgan 1889); *Aspidiotus agrumincola* De Gregorio, 1915; *Chrysomphalus dictyospermi agrumicola* De Gregorio, 1915; *Chrysomphalus arecae* (Newstead 1893); *Chrysomphalus jamaicensis* (Cockerell 1894); *Chrysomphalus castigatus* Mamet, 1936; *Aspidiotus agrumincola* (Cockerell 1894).

***Chrysomphalus pinnulifer* (Maskell 1891)** (Fig. 3F) (Maskell 1891; Chamberlin 1927)

Original spelling on label: *Chrysomphalus pinnulifer* Maskell. (1 slide)

Distribution: Thailand (Ubon).

Host plant: Lauracea.

Syn.: *Diaspis pinnulifera* Maskell, 1891; *Chrysomphalus dictyospermi pinnulifera* (Maskell 1891); *Chrysomphalus pinnuliferus* (Maskell 1891); *Aspidiotus* (*Chrysomphalus*) *pinnulifera* (Maskell 1891); *Aspidiotus pinnuliferus* (Maskell 1891).

***ClavAspidiotus tayabanus* (Cockerell 1905)** (Fig. 4A) (Takahashi 1933; McKenzie 1939; Takagi 1974)

Original spelling on label: *Chrysomphalus tayabanus* Ckll. (2 slides)

Distribution: Taiwan (Taito).

Syn.: *Aspidiotus tayabanus* Cockerell, 1905; *Aonidiella tayabana* (Cockerell 1905); *Chrysomphalus tayabanus* (Cockerell 1905); *Clavaspis tayabanus* (Cockerell 1905).

***Cryptoparlatorea pini* Takahashi, 1938** (Fig. 4B) (Takahashi 1938; Ben-Dov & Miller 2010)

Original spelling on label: *Cryptoparlatoria pini* Takah. (19 slides)

Distribution: India (Manali).

Host plant: *Pinus* sp..

Note: Syntypes, female [collected from India: Himachal Pradesh, Manali, Punjab, on *Pinus* sp., 18-VIII-1937 by Mr. Ansari (#25-1-1-1–#25-1-4-4)].

***Cryptoparlatoreopsis longispina* (Takahashi 1939)** (Fig. 4C) (Takahashi 1939; Borchsenius 1966; Ben-Dov & Miller 2010)

Original spelling on label: *Aonidia longispina* Takah. (6 slides)

Distribution: India (Bannu: Nurar Road).

Host plant: *Ziziphus mauritiana*.

Note: Syntypes, female [collected from India:

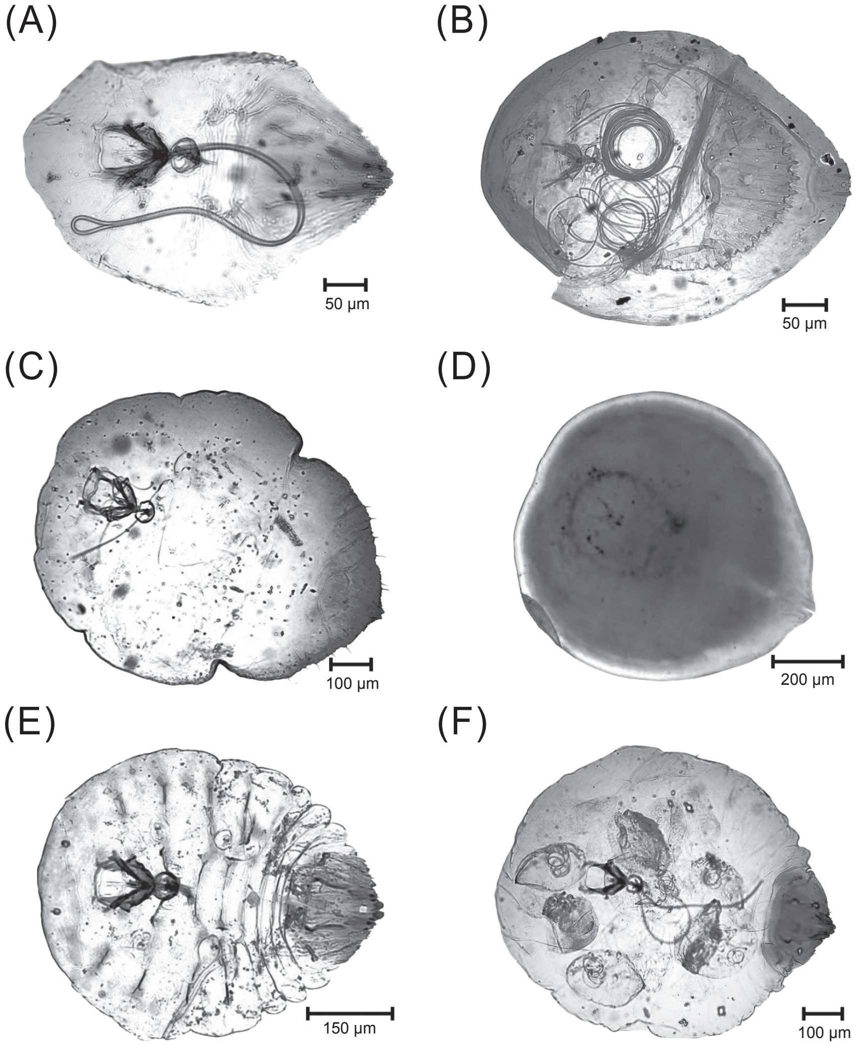

Fig. 4. (A) *Clavaspidiotus tayabanus* (Cockerell 1905); (B) *Cryptoparlatorea pini* (Takahashi 1938); (C) *Cryptoparlatoreopsis longispina* (Takahashi 1939); (D) *Diaonidia cinnamomi* (Takahashi 1936); (E) *Diaspidiotus makii* (Kuwana 1932); and (F) *Diaspidiotus perniciosus* (Comstock 1881).

Punjab, Bannu, on "Ber." 16-VIII-1938 by Ansari ([#]15-2-2-1–15-2-3-1)].

***DiAonidia cinnamomi* (Takahashi 1936)** (Fig. 4D)

(Takahashi 1936; Borchsenius 1966; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Aonidia cinnamomi* Takah. (1 slide)

Distribution: Taiwan (Takao: Chushinron near Rokki).

Host plant: *Cinnamomum* sp..

Syn.: *Aonidia cinnamomi* Takahashi, 1936; *Gymnaspis cinnamomi* Takahashi, 1936.

Note: Syntype, female [collected from Taiwan: Chushinron near Rokki, Takao Prefecture, on *Cinnamomum* sp., 19-V-1935 by R. Takahashi ([#]15-2-1-1)].

***DiAspidiotus makii* (Kuwana 1932)** (Fig. 4E)

(Kuwana 1932; Borchsenius 1966)

Original spelling on label: *Aspidiotus makii*

Kuw. (2 slides)

Distribution: Japan (Loochoo: Naha).

Host plant: *Pinus* sp..

Note: Specimens, [collected from Japan: Naha, Loochoo, on *Pinus* sp., IV-1930 by R. Takahashi (#17-2-2-1–17-2-2-2)].

***DiAspidiotus perniciosus* (Comstock 1881)** (Fig. 4F) (Comstock 1881; Tao 1989; Cockerell 1899a;Wong et al. 1999)

Original spelling on label: *Forbesaspis perniciosus* Comst. (5 slides)

Distribution: India (Parachanar); Korea.

Host plant: *Malus domestica*.

Syn.: *Aspidiotus perniciosus* Comstock, 1881; *Aonidia fusca* Maskell, 1895; *Aspidiotus albopunctatus* Cockerell, 1896; *Aonidiella fusca* (Maskell 1895); *Aonidiella perniciosa* (Comstock 1881); *Aspidiotus* (*DiAspidiotus*) *andromelas* Cockerell, 1897; *Aspidiotus* (*DiAspidiotus*) *perniciosus albopunctatus* (Comstock 1881); *Aspidiotus* (*DiAspidiotus*) *perniciosus* (Comstock 1881); *DiAspidiotus perniciosus andromelas* (Comstock 1881); *Aonidiella andromelas* Cockerell, 1897; *Aspidiotus perniciosus albopunctatus*; Cockerell, 1897; *Aspidiotus perniciosus andromelas* (Comstock 1881); *Aspidiotus* (*DiAspidiotus*) *perniciosus* (Comstock 1881); *Aspidiotus* (*Hemiberlesia*na) *perniciosus* (Comstock 1881); *Aspidiotus* (*Comstockaspis*) *perniciosus* (Comstock 1881); *QuadrAspidiotus perniciosus* (Comstock, 1881).

***DuplAspidiotus tesseratus* (Grandpré & Charmoy 1899)** (Fig. 5A) (Ferris 1938)

Original spelling on label: *DuplAspidiotus tessellatus* de Charmoy. (1 slide)

Distribution: Malaysia (Malaya: Serdang).

Host plant: *Bertholletia excelsa*.

Syn.: *Aspidiotus* (*DiAspidiotus*) *tesseratus* Grandpré & Charmoy, 1899; *Aspidiotus tesseratus* (Grandpré & Charmoy 1899); *PseudAonidia tesserata* (Grandpré & Charmoy 1899); *PseudAonidia oreodoxae* Rutherford, 1914; *LattAspidiotus oreodoxae* (Rutherford 1914); *LattAspidiotus tesserata* (Grandpré & Charmoy 1899); *PseudAonidia subtesserata* Green & Laing, 1923; *DuplAspidiotus subtesseratus* (Green & Laing 1923); *PseudAonidia* (*DuplAspidiotus*) *tesserata* (Grandpré & Charmoy 1899).

***DynAspidiotus pseudomeyeri* (Kuwana 1932)** (Fig. 5B) (Kuwana 1932; Takahashi & Takagi 1957)

Original spelling on label: *Aspidiotus pseudomeyeri* Kuw. (1 slide)

TARI specimens Distribution: Japan (Kagawa-ken).

Host plant: *Chamaecyparis obtuse*.

Syn.: *Aspidiotus pseudomeyeri* Kuwana, 1932; *TsugAspidiotus pseudomeyeri* (Kuwana 1932); *Nuculaspis pseudomeyeri* (Kuwana 1932).

***Furcaspis oceanica* Lindinger, 1909** (Fig. 5C) (Lindinger 1909; Borchsenius 1966)

(1) **Original spelling on label:** *Aspidiotus oceanica* Lind. (2 slides)
Distribution: USA (Saipan).

(2) **Original spelling on label:** *Chrysomphalus saipanensis* Sasaki. (6 slides)
Distribution: Micronesia (Caroline Isls.: Kusai); Palau (Korror).

(3) **Original spelling on label:** *Furcaspis oceanica* Lindg. (18 slides)
Distribution: Marshall Isls. (Jaluit-Imej; Watje); Micronesia (Caroline Isls.: Kusaie: Lelo; Ponape: Matalanim-Nipit, Paliker; Truk: Fefan, Fefan Mesa-Urunna, Pata Sabote, Sabote, Teiso Esaki); Palau Isls. (Kajangl;

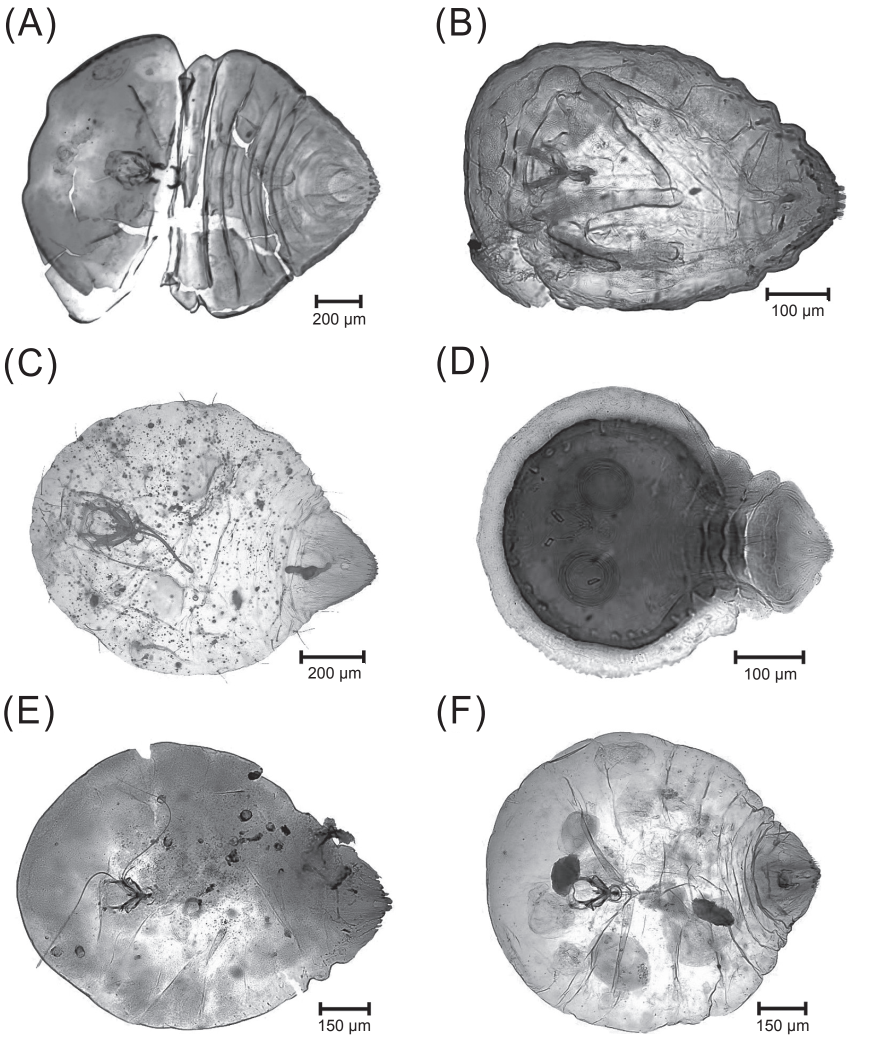

Fig. 5. (A) *Duplaspidiotus tesseratus* (Grandpré & Charmoy 1899); (B) *Dynaspidiotus pseudomeyeri* (Kuwana 1932); (C) *Furcaspis oceanica* (Lindinger 1909); (D) *Greeniella lahoarei* (Takahashi 1931); (E) *Hemiberlesia chipponsanensis* (Takahashi 1935); and (F) *Hemiberlesia lataniae* (Signoret 1869).

Korror).

Host plant: *Clinostigma ponapensis*, *Cocos nucifera*, *Musa* sp., Palm, *Pandanus* sp., *Roystonea regia*, *Nypa fruticans*.

Syn.: *Aspidiotus oceanica* Lindinger, 1909; *Furcaspis oceanica* (Lindinger 1909); *SpinAspidiotus oceanicus* (Lindinger 1909); *Chrysomphalus saipanensis* Sasaki, 1935; *Furcaspis saipanensis* (Sasaki 1935).

***Greeniella lahoarei* (Takahashi 1931)** (Fig. 5D) (Takahashi 1931a; Borchsenius 1966; Ben-Dov & Miller 2010)

Original spelling on label: *Aonidia lahoarei* Takah. (8 slides)

Distribution: Taiwan (Nantou: Suisha; Taihoku: Sozan).

Host plant: *Eugenia* sp., *Syzygium buxifolium*.

Syn.: *Aonidia* (*Greeniella*) *lahoarei* Takahashi, 1931.

Note: Syntypes, female (collected from Taiwan: Sozan, Suisha, on *Eugenia* sp., 26-

I-1930 by E. Kurosawa (#15-1-2-1–3)).

***Hemiberlesia chipponsanensis* (Takahashi 1935)** (Fig. 5E) (Takahashi 1935; Borchsenius 1966; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Aspidiotus chipponensis* Takah. (1 slide)

Distribution: Taiwan (Taito: Chipponsan).

Host plant: *Rhododendron metternichii*.

Syn.: *Aspidiotus chipponsanensis* Takahashi, 1935.

Note: Syntype, female (collected from Taiwan: Taito Province, Chipponsan, on *Rhododendron* sp., 23-III-1934 by R. Takahashi (#16-3-4-1)).

***Hemiberlesia lataniae* (Signoret 1869)** (Fig. 5F) (Cockerell 1905; Tao 1989; Wong *et al*. 1999)

Original spelling on label: *Aspidiotus lataniae* Signt. (31 slides)

Distribution: India (Bangalore); Malaysia (Malaya: Kuala Lumpur); Micronesia (Caroline Isls.: Truk: Toloas; Yap: Okao); Taiwan (Bokoto; Heito: Heito, Kuraru; Hualien: Karenko; Keelung; Taihoku: Maruyama, Taihoku; Taito; Takao); Thailand (Chiengmai; Mt. Sutep) USA (Saipan: Garapan, Maansha, Saipan).

Host plant: *Areca catechu*, *Averrhoa carambola*, *Barringtonia* sp., *Casuarina* sp., *Citrus* sp., *Chrysolidocarbus* sp., *Cycas* sp., *Diospyros discolor*, *Erythrodes latifolia*, *Gladiolus* spp., *Hibiscus* sp., Leguminosae, *Mangifera indica*, *Manilkara zapota*, *Pinus* sp., *Psidium* sp., *Samanea saman*, *Sandal* sp., *Thea* sp., *Vitis labrusca*.

Syn.: *Aspidiotus lataniae* Signoret, 1869; *Aspidiotus cydoniae* Comstock, 1881; *Aspidiotus punicae* Cockerell, 1893; *Aspidiotus diffinis lateralis* Cockerell, 1894; *Aspidiotus cydoniae tecta* Maskell, 1897; *Aspidiotus implicatus* Maskell, 1897; *Aspidiotus* (*Hemiberlesia*) *cydoniae* (Maskell 1897); *Aspidiotus* (*Hemiberlesia*) *crawii* Cockerell, 1897; *Aspidiotus* (*DiAspidiotus*) *punicae* (Cockerell 1893); *Aspidiotus* (*DiAspidiotus*) *greenii* Cockerell, 1897; *Aspidiotus* (*Aspidiotus*) *lataniae* (Signoret 1869); *Aspidiotus* (*EvAspidiotus*) *lataniae* (Signoret 1869); *Aspidiotus* (*EvAspidiotus*) *punicae* (Cockerell 1893); *Aspidiotus* (*EvAspidiotus*) *cydoniae* (Comstock 1881); *Hemiberlesia crawii* (Cockerell 1897); *Aspidiotus* (*EvAspidiotus*) *crawii* (Cockerell 1897); *Aspidiotus* (*EvAspidiotus*) *cydoniae tectus* (Maskell 1897); *Aspidiotus* (*EvAspidiotus*) *greenii* (Cockerell 1897); *Aspidiotus* (*EvAspidiotus*) *implicatus* (Maskell 1897); *Aspidiotus* (*Hemiberlesia*) *lataniae* (Signoret 1869); *Aspidiotus cydoniae crawii* (Comstock 1881); *Aspidiotus cydoniae punicae* (Comstock 1881); *Hemiberlesia lataniae* (Signoret 1869); *Aspidiotus cydoniae crawi* (Comstock 1881); *Aspidiotus cydoniae greeni* (Comstock 1881); *Aspidiella punicae* (Cockerell 1893); *Marlattaspis implicata* (Maskell 1897); *DiAspidiotus lataniae* (Signoret 1869); *Aspidiotus aspleniae* Sasaki, 1935; *DiAspidiotus* (*Aspidiotus*) *lataniae* (Signoret 1869); *Aspidiotus tectus* (Maskell 1897); *Hemiberlesia implicatus* (Maskell 1897).

***Hemiberlesia palmae* (Cockerell 1893)** (Fig. 6A) (Cockerell 1893b; Ferris 1938; Chou 1985)

Original spelling on label: *Aspidiotus javanensis* Kuwana. (8 slides)

Distribution: Fiji (Toverua); Japan (Honshu: Tokyo); Micronesia (Caroline Isls.:

Ponape: Kolonia); Palau Isls. (Koror).

Host plant: *Artocarpus communis*, *Avocado-pear* sp., *Cocos* sp..

Syn.: *Aspidiotus palmae* Morgan, 1893; *Aspidiotus* ***palmae*** Cockerell, 1893; *Aspidiotus* (*Hemiberlesia*) *palmae* (Cockerell 1893); *Aspidiotus* (*EvAspidiotus*) *palmae* (Cockerell 1893); *Aspidiotus unguiculatus* Leonardi, 1914; *Furcaspis palmae* (Cockerell 1893); *Aspidiotus javanensis* Kuwana in Kuwana & Muramatsu, 1931; *Abgrallaspis palmae* (Cockerell 1893); *Borchseniaspis palmae* (Cockerell 1893).

Hemiberlesia rapax **(Comstock 1881)** (Fig. 6B)

(Comstock 1881; Ferris 1938; Bodenheimer 1949; Tao 1989)

Original spelling on label: *DiAspidiotus camelliae* (Signoret 1869) (6 slides)

Distribution: Taiwan (Nantou: Onoe near Musha).

Syn.: *Aspidiotus acuminatus* Targioni Tozzetti,

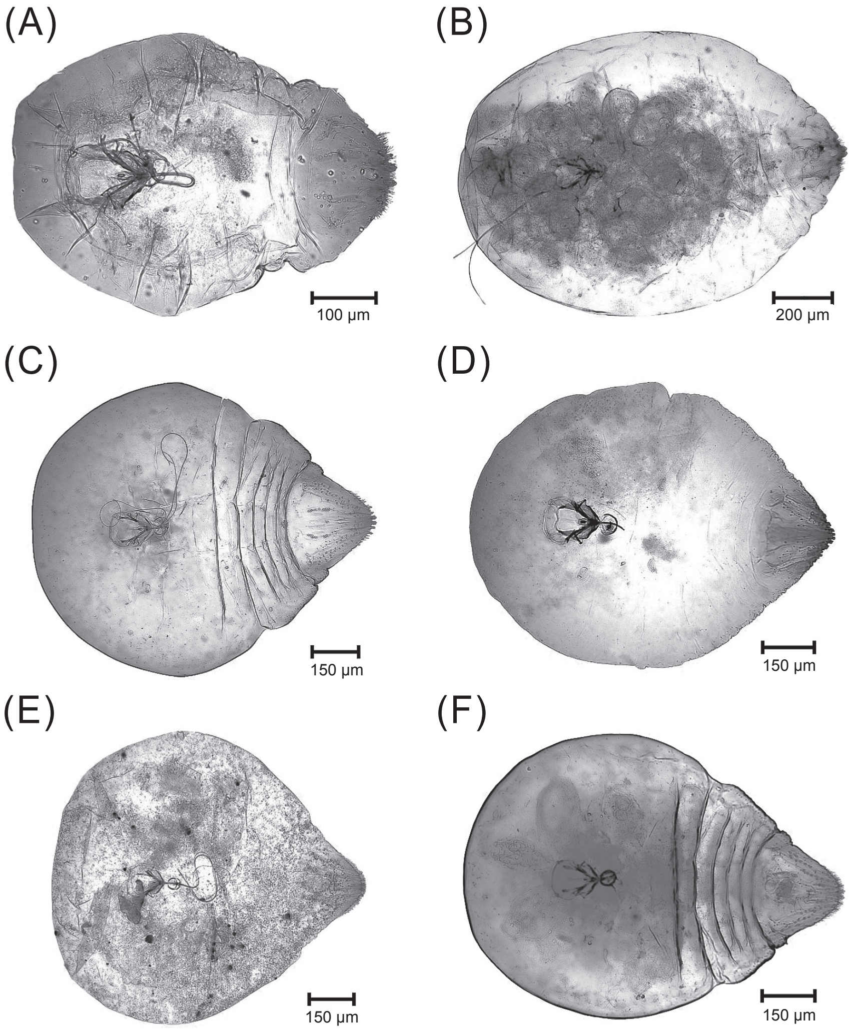

Fig. 6. (A) *Hemiberlesia palmae* (Cockerell 1893); (B) *Hemiberlesia rapax* (Comstock 1881); (C) *Lindingaspis rossi* (Maskell 1892); (D) *Melanaspis bromiliae* (Leonardi 1899); (E) *Octaspidiotus machili* (Takahashi 1931); and (F) *Octaspidiotus stauntoniae* (Takahashi 1933).

1881; *Aspidiotus rapax* Comstock, 1881; *Aspidiotus evonymi* Targioni Tozzetti, 1888; *Aspidiotus flavescens* Green, 1890; *DiAspidiotus camelliae* (Signoret 1869); *Diaspis circulata* Green, 1896; *Aspidiotus* (*Hemiberlesia*) *tricolor* Cockerell, 1897; *Aspidiotus lucumae* Cockerell, 1899; *Hemiberlesia argentina* Leonardi, 1911; *Hemiberlesiana camelliae* (Signoret 1869).

Lindingaspis rossi **(Maskell 1892)** (Fig. 6C) (Maskell 1892; Leonardi 1897; Ferris 1938; Tao 1989)

Original spelling on label: *Chrysomphalus rossi* (Maskell 1892) (13 slides)

Distribution: Taiwan (Nantou: Gyochi; Heito; Shintiku: Komoko; Taihoku; Tainan: Shokwa; Taito: Taito, Toran; Takao).

Host plant: *Aalbizia lucida*, *Euonymus japonicas*, *Ficus nipponica*, *Kandelia obovata*, *Mangifera indica*, *Palm, Pyracantha* sp., *Scolopia* sp., *Sedum alboroseum*, *Vitis* sp..

Syn.: *Aspidiotus rossi* Maskell, 1892; *Chrysomphalus rossi* (Maskell 1892); *Aspidiotus* (*Chrysomphalus*) *rossi* (Maskell 1892); *Melanaspis rossi* (Maskell 1892); *Aonidiella subrossi* Laing, 1929; *Chrysomphalus niger* Laing, 1929; *Melanaspis subrossi* (Maskell 1892).

Melanaspis bromiliae **(Leonardi 1899)** (Fig. 6D) (Fernald 1903; Lindinger 1932; Deitz & Davidson 1986)

(1) **Original spelling on label:** *Chrysomphalus bromeliae* Newst. (2 slides)

Distribution: Taiwan (Heito: Ropi near Heito).

(2) **Original spelling on label:** *Pseudischnaspis anassarum* Lindg. (3 slides)

Distribution: Micronesia (Caroline Isls.: Yap: Nif); Palau (Korror).

Host plant: *Ananas comosus*.

Syn.: *Aonidiella bromiliae* Leonardi, 1899; *Aspidiotus bromiliae* (Leonardi 1899); *Pseudischnaspis bromeliae* (Leonardi 1899); *Chrysomphalus* (*Melanaspis*) *bromeliae* Fernald, 1903.

OctAspidiotus machili **(Takahashi 1931)** (Fig. 6E) (Takahashi 1931b; Takagi 1984; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Aspidiotus machili* Takah. (1 slide)

Distribution: Taiwan (Taihoku: Sozan).

Host plant: *Machilus* sp..

Syn.: *Aspidiotus machili* Takahashi, 1931; *MetAspidiotus machili* (Takahashi 1931).

Note: Syntype, female [collected from Taiwan: Sozan, on *Machilus* sp., 21-VI-1931 by R. Takahashi (#17-2-2-4)].

OctAspidiotus stauntoniae **(Takahashi 1933)** (Fig. 6F) (Takahashi 1933; Takagi 1984; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Aspidiotus stauntoniae* Takah. (2 slides)

Distribution: Taiwan (Taihoku: Shinten).

Host plant: *Stauntonia obovatifolia*.

Syn.: *Aspidiotus stauntoniae* Takahashi, 1933; *MetAspidiotus stauntoniae* (Takahashi 1933).

Note: Syntypes, female [collected from Taiwan: Shinten, on *Stauntonia obovatifolia*, 16-X-1932 by R. Takahashi (#17-5-4-1–2)].

PseudAonidia curculiginis **(Green 1904)** (Fig. 7A) (Green 1904; Marlatt 1908; Lindinger 1909; Williams & Miller 2007)

Original spelling on label: *Furcaspis curculiginis* Green. (1 slide)

Distribution: Malaysia (Malaya: Fanting Sqmpah).

Host plant: *Gardenia* sp..

Syn.: *Aspidiotus* (*PseudAonidia*) *curculiginis* Green, 1904; *PseudAonidia circuliginis* (Green); *Furcaspis curculiginis* (Green 1904); *StringAspidiotus curculiginis* (Green 1904); *Aspidiotus curculiginis* (Green 1904).

PseudAonidia duplex **(Cockerell 1896)** (Fig. 7B)
(Cockerell 1896; Fernald 1903; Tao 1989; Wong *et al*. 1999)

Original spelling on label: *PseudAonidia duplex* Ckll. (8 slides)

Distribution: Japan (Honshu: Kobe; Wakayama); Taiwan (Taihoku; Takao; Toen: Heichin, Heitin).

Host plant: *Camellia sinensis*, *Cinnamomum camphora*, *Citrus* sp..

Syn.: *Aspidiotus theae* Maskell, 1891; *Aspidiotus duplex* Cockerell, 1896; *Aspidiotus* (*PseudAonidia*) *duplex* (Cockerell 1896); *Aspidiotus* (*PseudAonidia*) *theae* (Cockerell 1891); *Aspidiotus* (*EvAspidiotus*) *duplex* (Cockerell 1896); *Aspid-*

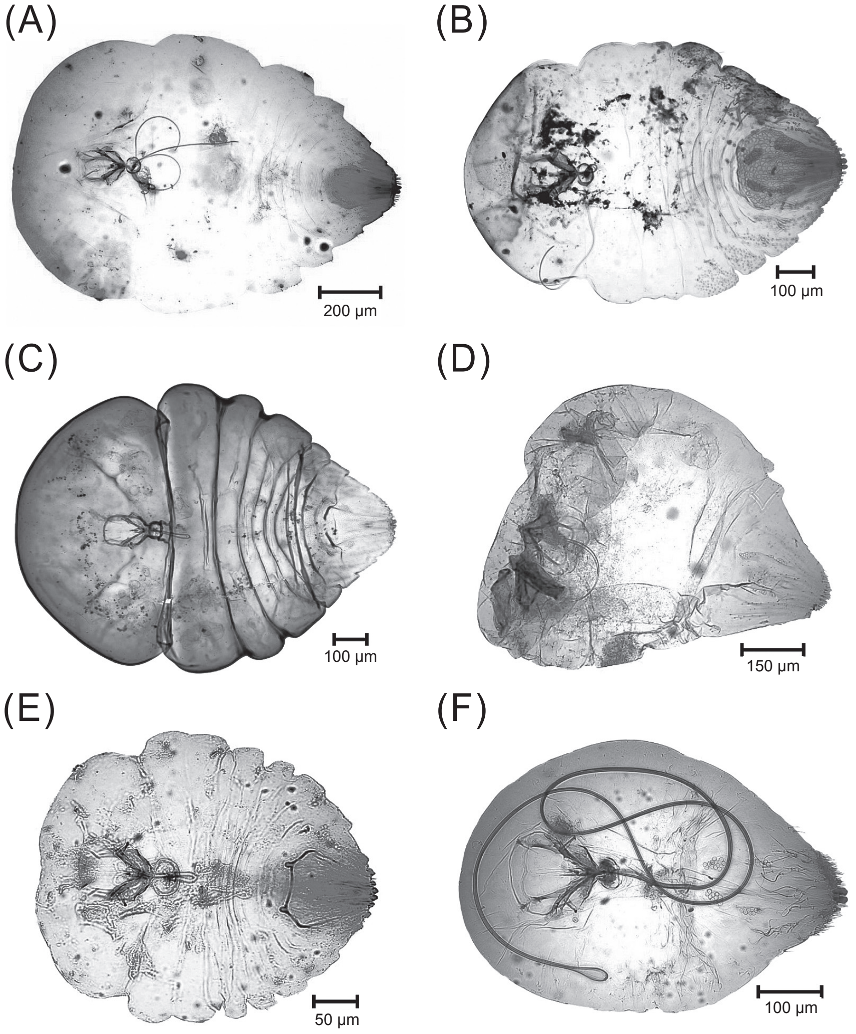

Fig. 7. (A) *Pseudaonidia curculiginis* (Green 1904); (B) *Pseudaonidia duplex* (Cockerell 1896); (C) *Pseudaonidia trilobitiformis* (Green 1896); (D) *Selenomphalus euryae* (Takahashi 1931); (E) *Semelaspidus mangiferae* (Takahashi 1939); and (F) *Taiwanaspidiotus shakunagi* (Takahashi 1935).

iotus (*EvAspidiotus*) *theae* (Cockerell 1891); *Aspidiotus theae rhododendri* Green, 1900; *PseudAonidia rhododendri* (Green 1900); *PseudAonidia rhododendri thearum* Fernald, 1903.

***PseudAonidia trilobitiformis* (Green 1896)** (Fig. 7C) (Green 1896a; Cockerell 1899a; Tao 1989; Wong et al. 1999)

Original spelling on label: *PseudAonidia trilobitiformis* Green. (25 slides)

Distribution: Cambodia (Angkor); Indonesia (Java); Taiwan (Bokoto: Mako; Heito: Garanbi; Kagi; Taichu: Kahodai; Taihoku: Sozan, Taihoku, Taipei; Taito: Kanaron, Kotosho); Thailand (Ubon).

Host plant: *Artocarkus* sp., *Bischoffia* sp., *Buxus microphylla*, *Calophyllum inophyllum*, *Citrus* sp., *Fhenacaspus* sp., *Ficus retusa*, *Ficus* sp., *Hydrangea* sp., *Machilus* sp., *Mangifera indica*, *Murraya exotica*, *Murraya paniculata*, *Quercus* sp., *Scolopia oldhamii*.

Syn.: *Aspidiotus trilobitiformis* Green, 1896; *Aspidiotus trilobitiformis* (Green 1896); *Aspidiotus darutyi* Charmoy, 1898; *Aspidiotus* (*EvAspidiotus*) *trilobitiformis* (Green 1896); *PseudAonidia trilobitiformis darutyi* (Green 1896); *PseudAonidia darutyi* (Charmoy 1898).

***Selenomphalus euryae* (Takahashi 1931)** (Fig. 7D) (Takahashi 1931b; Lindinger 1943; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Aspidiotus euryae* Takahashi, 1931 (4 slides)

Distribution: Taiwan (Giran: Taiheisan, Taihezan; Nantou: Suisha; Taichu: Rirei).

Host plant: *Eurya* sp..

Syn.: *Aspidiotus euryae* Takahashi, 1931; *Selenaspidus euryae* (Takahashi 1931).

Note: Syntype, female [collected from Taiwan: Taihezan, Suisha, on *Eurya* sp., 21-V-1921 by R. Takahashi (#17-2-1-3)].

***Semelaspidus mangiferae* Takahashi, 1939** (Fig. 7E) (Takahashi 1939; Tao 1989; Ben-Dov & Miller 2010)

Original spelling on label: *Semelaspidus mangiferae* Takah. (1 slide)

Distribution: Philippine.

Host plant: *Mangifera indica*.

Note: Syntype, female [collected from Philippines: on *Mangifera indica*, III-1933 by R. Takahashi (#20-5-4-1)].

***TaiwanAspidiotus shakunagi* (Takahashi 1935)** (Fig. 7F) (Takahashi 1935; Takagi 1969; Tao 1989)

Original spelling on label: *Aspidiotus shakunagi* Takah. (2 slides)

Distribution: Taiwan (Taito: Chipponsan).

Host plant: *Cerbera manghas*, *Rhododendron Bergian*.

Syn.: *Aspidiotus shakunagi* Takahashi, 1935.

Note: Syntypes [collected from Taiwan: Taito Province, Chipponsan, on *Rhododendron* sp., 23-III-1934 by R. Takahashi (#17-5-3-1–2)].

DISCUSSION

Based on the modern taxonomic system, the number of Aspidiotinae species collected and deposited at TARI Insect Collection is 42. The present paper could be a database to set up the online information of scientific name, synonyms, host plants, distribution and digital archives through Digital Insect of TARI website (http://digiins.tari.gov.tw/) which will meet of the requirements of both basic and applied works and will be helpful to taxonomic researchers, quarantine inspector inspections and farmers in the future.

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農業試驗所昆蟲標本館圓盾介殼蟲亞科 (半翅目、胸喙亞目、介殼蟲總科、盾介殼蟲科) 標本名錄

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摘要

陳淑佩、翁振宇、吳文哲。2013。農業試驗所昆蟲標本館圓盾介殼蟲亞科 (半翅目、 胸喙亞目、介殼蟲總科、盾介殼蟲科) 標本名錄。台灣農業研究 62(4):300–320。

本文羅列 23 屬 42 種 (391 片玻片標本) 之農業試驗所館藏之圓盾介殼蟲亞科 [半翅目 (Hemiptera) 胸喙亞目 (Sternorrhyncha) 介殼蟲總科 (Coccoidea) 盾介殼蟲科 (Diaspididae)] 名錄，其中亦包含異名、寄主植物、採集分佈及標本形態圖等資料。

關鍵詞：盾介殼蟲、農業試驗所、館藏標本。

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Changes of Climatic Variables during Grain-filling Stage Affect Yield and Quality of Rice Cultivars Bred from Different Regions in Taiwan

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Abstract

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Changes in climatic variables during grain-filling stage are known to affect yield and quality of rice at harvest. However, there exist differential effects on cultivars that bred from varied regions with diversified genetic contexts and environmental conditions. To effectively response to different conditions emerging from changing climate for a stable food supply, it is necessary to further clarify the underlying mechanisms of climatic effects. This study was conducted in years from 2008 to 2010 with a total of 29 cropping seasons based on 24 Solar Terms calendar. Four rice (*Oryza sativa* L.) cultivars bred from different regions in Taiwan were selected for experiments, and weather data recorded from nearby meteorological station were used for analyses. The mean values of daily mean air temperature, daily maximum air temperature, daily minimum air temperature, day-night temperature difference, daily sunshine hours and daily irradiance during grain-filling stage were calculated as six climatic variables of MDMAT, MDMAXAT, MDMINAT, MDNTD, MDSH and MDIR, respectively. Results showed that temperature variables had a greater weight than irradiance and sunshine hour, and amylose, transparency and head rice rate of milled grains were the quality traits affected by temperature changes. The curvilinear relationships were significant only for 'TY4' in the first-half years (FHY), but were significant for all cultivars in the second-half years (SHY). Temperature variables also had an effect on grain yield, the convex curve patterns indicating that there were optimal temperature regimes. Analyses on the pooled data of 4 cultivars indicated that rice yields reached the plateau at MDMINAT of 23℃, MDMAT of 26.5℃ and MDMAXAT of 31℃ in FHY, and were at MDMINAT of 20℃, MDMAT of 24℃ and MDMAXAT of 29℃ in SHY. Both MDNTD and MDIR had a quadratic relationship with quality traits in SHY, and similar relationship was found in MDNTD with grain yield in SHY. This study demonstrated the differential effects of climatic variables during grain-filling stage on chemical components, physical characters and yield of rice grains in cultivars bred from varied regions. Temperature variables above the threshold values that obtained the maximum yield result in a decline of yield production.

Key words: Grain-filling stage, Climatic variable, Cultivar bred from varied regions, Rice quality, Rice yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major food crops cultivated worldwide including Taiwan. As a traditional staple food, its cultivation hectarage reached 260,000 ha with a total of 1.37 million ton in 2012 (COA 2012). The

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subtropical climate plus sufficient technical support enable a two-crop production system to ensure an abundant food supply for local demand. However, climate scenarios are quite opposite in these two crops. An increasing air temperature and solar radiation occurs in the first cropping season, while an inverse situation take place in the second cropping season making a differential outcome of climatic effects on yield and quality (Chu & Lu 1984; Liu *et al*. 1984). During grain-filling stage of first crop, rice plants often suffered high temperature impairments to result a high ratio of chalky grains; whereas, long period hot weather in tillering stage of second crop frequently reduce effective tillers. The low irradiance and sunshine hours during the late grain filling also limit the supplement of assimilates from photosynthesis, thereby restricting capacity of grain filling and thus lowering down rice yield of second crop by 20–25% relative to first crop (Lin & Hung 1979; Lin *et al*. 1979; Liu & Hsieh 1984; Lur *et al*. 2009).

Four aspects are mostly concerned in terms of rice quality, i.e., milling characteristics, grain appearance, nutritional value and cooking performance. Each part contributes to taste and eating nature of rice. For grain appearance, generally shape, uniformity, transparency and chalkiness (or whiteness) of grains are four important physical characters being taken into account. As for nutritional value, protein, fatty acids, starch, vitamins, minerals and water are the most interested chemical components (Kim *et al*. 1994; Hong *et al*. 2000; Hsu *et al*. 2000; Choi 2002). In addition to cultivational practices taken along the growing process, such as fertigation, irrigation and pests management, yield and qualiy of a rice crop are influenced by varietal genetic variability and environmental variation. Among environmental factors, temperature and radiatoin have been studied extensively (Tsai *et al*. 1984; Islam & Morison 1992; Lee *et al*. 2009; Ho *et al*. 2012). As literature indicated, high temperatures stimulate grain-filling rate and shorten grain-filling period to increase protein content and chalky grains but decrease amylose and grain weight, as well as to enhance respiration consumption at night and bring about reduced pollen germination percentage and sterility of spikelets (Zakaria *et al*. 2002; Hirai *et al*. 2003; Morita *et al*. 2004; Yamakawa *et al*. 2007; Mohammed & Tarpley 2010; Kim *et al*. 2011). Low temperatures occur during grain-filling would raise proportions of immature, green, abnormal and dead grains and contents of crude protein and amylose in endosperm of rice grains (Huang & Lur 2000; Ahmed *et al*. 2008). As a result, temperatures higher than 30℃ or lower than 20℃ would not only reduce milling quality, appearance and yield of rice grains but also lessen their nutritional value, eating and cooking performance. Some experimental results further revealed that 1℃ increase at night would cut down 10% yield at harvest (Kukla & Karl 1993; Easterling *et al*. 1997; Peng *et al*. 2004), which became more evident when rate of temperature increase was greater at night than in daytime.

Daily irradiance and sunshine hours also play a role in affecting rice yield and quality. Nagarajan *et al*. (2010) pointed out that there were positive relationships between irradiance and spikelet fertility, 1000-grain weight, harvest index and production of rice. Yang (1994) reported that under well-watered condition, grain yield of rice increased with the increasing cumulative daily irradiance and daily mean air temperature. Others (Tsai *et al*. 1984; Lur *et al*. 2006) showed that photosynthetic rate was inhibited at low irradiance so that grain-filling was jeopardized to cause lower grain-filling rate and 1000-grain weight.

The 2007 IPCC Fourth Assessment Report on climate change disclosed an increasing trend of air temperature in the future (IPCC 2007). To safeguard a stable and available food supply system, it is necessary to continue vigorous efforts of research against negative impacts emerging from a changing climate. Although changes in climatic variables during grain-filling stage of rice have been known to affect yield and quality, the differential effects on cultivars that bred from varied regions with diversified genetic contexts and environmental conditions are less explored. To be effectively response to different conditions rising from rapid climate change, the underlying mechanisms of climatic effects should be studied in more detail. The present research was carried out for the objectives as aforementioned.

MATERIALS AND METHODS

Cropping Seasons and Cultivation

Field experiments were conducted at the experimental farm of Taiwan Agricultural Research Institute (TARI; Wufeng, Taichung) in years from 2008 to 2010 with a total of 29 cropping seasons. Three-to-four-leaf stage rice seedlings were machined transplanted in a north-south direction based on 24 Solar Terms calendar, with the terms separated by 15 days apart along traditional East Asian lunisolar calendars. In the first-half year (seasons of First Crop), rice was planted 5 times (5 cropping seasons) taking solar term 'Lichun' (literally "start of spring" or "the first day of spring") as the center cropping season, and another 5 plantings adopted solar term 'Dashu' (literally "major heat" or "first day of mid-summer") as the center season in the second-half year (seasons of Second Crop) (Tables 1A and 1B). Rice cultivars, namely, 'TY4', 'TNG71', 'TK9' and 'KH145', bred from different regions in Taiwan were selected for experiments (Table 2), and weather data recorded from nearby agricultural meteorological station at TARI were used for analyses.

As shown in Table 2, these 4 selected rice cultivars were bred by different research institutions, located from the northern to the southern part of Taiwan, with diverse characteristics set by breeders. Similar management practices were applied to each cultivar in every cropping season so that growth and development of rice plants were mainly attributed to variation of climatic conditions for each cultivar. Multiple seedlings per hill were machine-transplanted in every cropping season, with a plot size of 0.1 ha (50 m × 20 m) for each cultivar that distributed randomly along the field. The soil pH was 5.36 and organic matter was 2.21%. The basal dose of fertilizer (Tai-Fei No.39, N : P : K = 12 : 18 : 12, granule, Taiwan Fertilizer Company, Kaohsiung, Taiwan) was applied at a rate of 200 kg ha^{-1} at 2–3 days before transplanting, followed by application of ammonium sulfate (21% N, granule, TFC, Kaohsiung, Taiwan) at a rate of 200 kg ha^{-1} at 1–2 weeks after transplanting (WAT). On 4–5 WAT, ammonium sulfate of 200 kg ha^{-1} was applied and Tai-Fei No.39 of 100 kg ha^{-1} was used at 1–2 weeks before heading as last dose. The herbicide 'Butachlor + Benthiocarb' (8% granule, 2.4 kg a.i ha^{-1}, Taiwan Ihara Chemical Co. Ltd., Taipei) was sprayed to control annual weeds upon transplanting and hand weeding was practiced 3–4 WAT. The Camellia sp. oil cake was sprayed over fields 1–2 times to control apple snails at a rate of 100 kg ha^{-1}, while 3% validamycin A (soluble concentrate, 0.036 L a.i. ha^{-1}, 1000×, Rich Country Chemical Corp., Taoyuan, Taiwan) and 10% Tecloftalam (wettable powder, 0.1 kg a.i. ha^{-1}, 1000×, Sinon Chemical Co., Ltd., Taichung, Taiwan) were applied to control sheath blight and bacterial

leaf blight, respectively. The 6% Cartap hydrochloride (granule, 1.8 kg a.i. ha^{-1}, Sinon Chemical Co., Ltd., Taichung, Taiwan) was applied when symptoms caused by rice leaf folder and stem borer occurred. For other pests, 40.64% Carbofuran (suspension concentrates, 0.49 L a.i. ha^{-1}, 1000×, Chia Yi Chemical Industry Co., Ltd., Tainan, Taiwan) was used.

Climatic Variables

A meteorological station designated for agricultural use and operated by TARI is situated within 500 meters away from the experimental fields. The mean values of daily mean air temperature (DMAT), daily maximum air temperature (DMAXAT), daily minimum air temperature (DMINAT), day-night temperature difference (DNTD), daily sunshine hours (DSH) and daily irradiance (DIR) during grain-filling stage were calculated to obtained the corresponding six climatic variables of MDMAT, MDMAXAT, MDMINAT, MDNTD, MDSH and MDIR, respectively. The regression analyses were performed to link and compare the differential effects of climatic variables on quality traits and grain yield.

Rice Quality Analysis (Physical Characters and Chemical Components)

At maturity of every cropping season, an area of 10 m^2 was harvested, with three replicates for each cultivar and the averaged value was used as representative for yield determination. After fresh weights taken and oven-dried at 40℃ for more than 72 h, dry weights were recorded at grain water content around 12–13% to estimate yields (Mg ha^{-1}) and analyze quality traits (Hsiao *et al*. 2009). Forty grains were randomly selected from each replication and viewed by a 5-fold enlarger (Model 66-SII, LPL Co. Ltd., Japan) to measure length and width of grains, and grain thickness was determined by a dial thickness gauge (Model SM540, TECLOCK Co., Japan). One thousand grain were also randomly selected to classify the proportions of dead, head and non-head rice by a grain color sorter (Model RGQI20A, Satake Co., Japan); the non-head rice included immature, damaged, chalky, green, discolored and cracked grains. The brightness of grains, in terms of transparency and whiteness, were determined by a milling meter (Model MM1C, Satake Co., Japan). The chemical components were analyzed by a near infrared grain tester (Model AN-820, Kett Co., Japan) to determine crude protein and amylose of milled grains and crude protein, amylose and free fatty acids of brown rice using the internal calibration curves for japonica rice.

Data Analysis and Statistics

The statistics of experimental data were conducted by the Statistical Analysis System (SAS version 9.1), while graphs were plotted by using the SigmaPlot software (version 10.0).

RESULTS AND DISCUSSION

To further understand how changes of climatic variables during grain-filling stage affect yield and quality of rice cultivars bred from different regions in Taiwan, this study adopted 24 Solar Terms calendar to plant rice and obtained sets of weather data from 29 cropping seasons in a 3-year experimental period. Based on the planting calendar and growth development (Tables 1 and 2), three climatic variables (i.e., MDMINAT, MDMAT and MDMAXAT) calculated from the data collected from the nearby weather station, for every cropping season and each rice cultivar, were found correlated with quality traits, while the other three variables had a less weight on the correlations (data not shown). Among them, changes in amylose and transparency of milled rice and head

Table 1A. Dates of transplanting (DT), heading (DHe) and harvest (DHa) and days required from heading to harvest of 29 cropping seasons for rice cultivar 'TY4' and 'TNG71' grown in 2008–2010.

Cropping season	DT	DHe	DHa	Cropping season	DT	DHe	DHa
TY4, 1st crop, 2008				2nd crop, 2008			
SS – 30[z]	na[y]	na	na	MH – 30	06/19/2008	08/21/2008	10/12/2008 (53)
SS – 15	01/16/2008	04/24/2008	06/09/2008 (47[x])	MH – 15	07/08/2008	09/01/2008	10/21/2008 (51)
SS	02/04/2008	05/02/2008	06/15/2008 (41)	MH	07/23/2008	09/16/2008	11/03/2008 (49)
SS + 15	02/19/2008	05/07/2008	06/19/2008 (44)	MH + 15	08/06/2008	09/28/2008	11/11/2008 (45)
SS + 30	03/06/2008	05/11/2008	06/23/2008 (44)	MH + 30	08/21/2008	10/14/2008	12/10/2008 (58)
1st crop, 2009				2nd crop, 2009			
SS – 30	12/24/2008	04/03/2009	05/21/2009 (49)	MH – 30	06/23/2009	08/20/2009	10/19/2009 (61)
SS – 15	01/20/2009	04/14/2009	05/30/2009 (47)	MH – 15	07/07/2009	08/30/2009	10/25/2009 (57)
SS	02/09/2009	04/27/2009	06/10/2009 (45)	MH	07/23/2009	09/17/2009	11/01/2009 (46)
SS + 15	02/19/2009	05/07/2009	06/18/2009 (43)	MH + 15	08/10/2009	09/29/2009	11/16/2009 (49)
SS + 30	03/05/2009	05/17/2009	06/25/2009 (40)	MH + 30	08/20/2009	10/12/2009	11/29/2009 (49)
1st crop, 2010				2nd crop, 2010			
SS – 30	12/31/2009	04/02/2010	05/17/2010 (46)	MH – 30	06/22/2010	08/15/2010	10/11/2010 (58)
SS – 15	01/14/2010	04/14/2010	05/25/2010 (42)	MH – 15	07/07/2010	09/06/2010	10/24/2010 (49)
SS	02/04/2010	04/21/2010	05/30/2010 (40)	MH	07/23/2010	09/15/2010	10/26/2010 (42)
SS + 15	02/23/2010	05/05/2010	06/17/2010 (44)	MH + 15	08/05/2010	09/29/2010	11/14/2010 (47)
SS + 30	03/04/2010	05/11/2010	06/22/2010 (43)	MH + 30	08/25/2010	10/15/2010	12/07/2010 (54)

Table 1A. Dates of transplanting (DT), heading (DHe) and harvest (DHa) and days required from heading to harvest of 29 cropping seasons for rice cultivar 'TY4' and 'TNG71' grown in 2008–2010. (continued)

Cropping season	DT	DHe	DHa	Cropping season	DT	DHe	DHa
TNG71, 1st crop, 2008				2nd crop, 2008			
SS – 30	na	na	na	MH – 30	06/19/2008	08/18/2008	10/07/2008 (51)
SS – 15	01/16/2008	04/21/2008	06/03/2008 (44)	MH – 15	07/08/2008	09/02/2008	10/21/2008 (50)
SS	02/04/2008	04/24/2008	06/05/2008 (43)	MH	07/23/2008	09/17/2008	11/03/2008 (48)
SS + 15	02/19/2008	05/06/2008	06/11/2008 (37)	MH + 15	08/06/2008	09/26/2008	11/10/2008 (46)
SS + 30	03/06/2008	05/08/2008	06/18/2008 (42)	MH + 30	08/21/2008	10/13/2008	12/03/2008 (52)
1st crop, 2009				2nd crop, 2009			
SS – 30	12/24/2008	04/06/2009	05/20/2009 (45)	MH – 30	06/23/2009	08/17/2009	10/12/2009 (57)
SS – 15	01/20/2009	04/17/2009	06/03/2009 (48)	MH – 15	07/07/2009	08/28/2009	10/22/2009 (56)
SS	02/09/2009	04/23/2009	06/09/2009 (48)	MH	07/23/2009	09/15/2009	10/29/2009 (45)
SS + 15	02/19/2009	05/05/2009	06/14/2009 (41)	MH + 15	08/10/2009	09/27/2009	11/12/2009 (47)
SS + 30	03/05/2009	05/14/2009	06/23/2009 (41)	MH + 30	08/20/2009	10/07/2009	11/18/2009 (43)
1st crop, 2010				2nd crop, 2010			
SS – 30	12/31/2009	03/31/2010	05/11/2010 (46)	MH – 30	06/22/2010	08/14/2010	10/10/2010 (58)
SS – 15	01/14/2010	04/12/2010	05/24/2010 (42)	MH – 15	07/07/2010	08/30/2010	10/20/2010 (52)
SS	02/04/2010	04/20/2010	05/26/2010 (40)	MH	07/23/2010	09/12/2010	10/20/2010 (39)
SS + 15	02/23/2010	05/02/2010	06/07/2010 (44)	MH + 15	08/05/2010	09/22/2010	11/07/2010 (47)
SS + 30	03/04/2010	05/09/2010	06/16/2010 (43)	MH + 30	08/25/2010	10/12/2010	12/01/2010 (51)

[z] SS: 'Lichun' (literally "start of spring" or "the first day of spring"). SS - 30 and SS - 15 are transplanting of rice seedlings at 30 and 15 days before 'Lichun'; SS + 15 and SS + 30 are 15 and 30 days after the date of 'Lichun'. MH: 'Dashu' (literally "major heat" or "first day of mid-summer"). MH – 30 and MH – 15 are transplanting of rice seedlings at 30 and 15 days before 'Dashu'; MH + 15 and MH + 30 are 15 and 30 days after the date of 'Dashu'.

[x] numbers in parentheses are days required from heading to harvest.

[y] na: not available.

Table 1B. Dates of transplanting (DT), heading (DHe) and harvest (DHa) and days required from heading to harvest of 29 cropping seasons for rice cultivar 'TK9' and 'KH145' grown in 2008–2010.

Cropping season	DT[y]	DHe	DHa	Cropping season	DT	DHe	DHa
TK9, 1st crop, 2008				2nd crop, 2008			
SS – 30[z]	na[y]	na	na	MH – 30	06/19/2008	08/24/2008	10/14/2008 (52)
SS – 15	01/16/2008	04/27/2008	06/10/2008 (45[x])	MH – 15	07/08/2008	09/08/2008	10/28/2008 (51)
SS	02/04/2008	05/05/2008	06/16/2008 (39)	MH	07/23/2008	09/21/2008	11/09/2008 (50)
SS + 15	02/19/2008	05/12/2008	06/22/2008 (42)	MH + 15	08/06/2008	10/02/2008	11/18/2008 (48)
SS + 30	03/06/2008	05/13/2008	06/25/2008 (44)	MH + 30	08/21/2008	10/21/2008	12/16/2008 (57)
1st crop, 2009				2nd crop, 2009			
SS – 30	12/24/2008	04/17/2009	05/26/2009 (40)	MH – 30	06/23/2009	08/19/2009	10/21/2009 (64)
SS – 15	01/20/2009	04/22/2009	06/08/2009 (48)	MH – 15	07/07/2009	09/01/2009	10/29/2009 (59)
SS	02/09/2009	04/30/2009	06/11/2009 (43)	MH	07/23/2009	09/22/2009	11/05/2009 (45)
SS + 15	02/19/2009	05/08/2009	06/17/2009 (41)	MH + 15	08/10/2009	10/02/2009	11/22/2009 (52)
SS + 30	03/05/2009	05/18/2009	06/25/2009 (39)	MH + 30	08/20/2009	10/14/2009	12/06/2009 (54)
1st crop, 2010				2nd crop, 2010			
SS – 30	12/31/2009	04/08/2010	05/18/2010 (41)	MH – 30	06/22/2010	08/19/2010	10/12/2010 (55)
SS – 15	01/14/2010	04/09/2010	05/25/2010 (37)	MH – 15	07/07/2010	09/07/2010	10/25/2010 (49)
SS	02/04/2010	04/21/2010	05/31/2010 (41)	MH	07/23/2010	09/14/2010	11/01/2010 (49)
SS + 15	02/23/2010	05/06/2010	06/14/2010 (40)	MH + 15	08/05/2010	10/04/2010	11/21/2010 (49)
SS + 30	03/04/2010	05/10/2010	06/21/2010 (43)	MH + 30	08/25/2010	10/19/2010	12/15/2010 (58)

Table 1B. Dates of transplanting (DT), heading (DHe) and harvest (DHa) and days required from heading to harvest of 29 cropping seasons for rice cultivar 'TK9' and 'KH145' grown in 2008–2010. (continued)

Cropping season	DTy	DHe	DHa	Cropping season	DT	DHe	DHa
KH145, 1st crop, 2008				2nd crop, 2008			
SS – 30	na	na	na	MH – 30	06/19/2008	08/20/2008	10/08/2008 (50)
SS – 15	01/16/2008	04/24/2008	06/05/2008 (43)	MH – 15	07/08/2008	09/05/2008	10/26/2008 (52)
SS	02/04/2008	05/02/2008	06/11/2008 (37)	MH	07/23/2008	09/19/2008	11/06/2008 (49)
SS + 15	02/19/2008	05/07/2008	06/18/2008 (43)	MH + 15	08/06/2008	09/30/2008	11/11/2008 (43)
SS + 30	03/06/2008	05/10/2008	06/23/2008 (46)	MH + 30	08/21/2008	10/18/2008	12/14/2008 (58)
1st crop, 2009				2nd crop, 2009			
SS – 30	12/24/2008	04/05/2009	05/24/2009 (50)	MH – 30	06/23/2009	08/18/2009	10/18/2009 (64)
SS – 15	01/20/2009	04/16/2009	06/05/2009 (51)	MH – 15	07/07/2009	08/31/2009	10/27/2009 (59)
SS	02/09/2009	04/23/2009	06/09/2009 (48)	MH	07/23/2009	09/18/2009	11/03/2009 (45)
SS + 15	02/19/2009	05/05/2009	06/14/2009 (41)	MH + 15	08/10/2009	10/01/2009	11/15/2009 (52)
SS + 30	03/05/2009	05/15/2009	06/24/2009 (41)	MH + 30	08/20/2009	10/09/2009	11/26/2009 (54)
1st crop, 2010				2nd crop, 2010			
SS – 30	12/31/2009	04/06/2010	05/16/2010 (41)	MH – 30	06/22/2010	08/15/2010	10/11/2010 (58)
SS – 15	01/14/2010	04/17/2010	05/24/2010 (38)	MH – 15	07/07/2010	09/06/2010	10/21/2010 (46)
SS	02/04/2010	04/22/2010	05/27/2010 (36)	MH	07/23/2010	09/17/2010	10/31/2010 (45)
SS + 15	02/23/2010	05/05/2010	06/07/2010 (34)	MH + 15	08/05/2010	10/02/2010	11/18/2010 (48)
SS + 30	03/04/2010	05/11/2010	06/16/2010 (37)	MH + 30	08/25/2010	10/18/2010	12/12/2010 (56)

[z] SS: 'Lichun' (literally "start of spring" or "the first day of spring"). SS - 30 and SS - 15 are transplanting of rice seedlings at 30 and 15 days before 'Lichun'; SS + 15 and SS + 30 are 15 and 30 days after the date of 'Lichun'. MH: 'Dashu' (literally "major heat" or "first day of mid-summer"). MH – 30 and MH – 15 are transplanting of rice seedlings at 30 and 15 days before 'Dashu'; MH + 15 and MH + 30 are 15 and 30 days after the date of 'Dashu'.
[x] numbers in parentheses are days required from heading to harvest.
[y] na: not available.

Table 2. The information about rice (*Oryza sativa* L.) cultivars selected for this study.

Rice cultivar	Institution release	Characteristics	Coordinate
Taoyuan No. 4 (TY4)	Taoyuan District Agricultural Research and Extension Station	Good eating quality; resistant to lodging; low on-site sprouting rate; low threshing rate; resistant to leaf blast (Chen *et al*. 2007)	121.028595 °E 24.952262 °N
Tainung No. 71 (TNG71)	Taiwan Agricultural Research Institute	Aromatic rice; good grain transparency; low chalky grains; better viscosity and elasticity of cooked rice (Lai *et al*. 2001)	120.695524 °E 24.027955 °N
Taikeng No. 9 (TK9)	Taichung District Agricultural Research and Extension Station	Good grain uniformity; good grain transparency; good grain appearance; good plant type; resistant to lodging; resistant to silk leaf blight, spot planthopper and white-backed planthopper (Hsu & Sung 1993)	120.533848 °E 24.000872 °N
Kaohsiung No. 145 (KH145)	Kaohsiung District Agricultural Research and Extension Station	Good milling quality; good grain transparence; low grain white belly; low crude protein content; good plant type; suitable for both crops (Chiu & Wu 2005)	120.524276 °E 22.709576 °N

rice ratio to variations of climatic variables were plotted and their regression relations were analyzed (Figs. 1–3). As indicated (Fig. 1), the relationships between amylose content and MDMINAT, MDMAT and MDMAXAT were best fitted to a quadratic function but only ‘TY4’ reached significant level ($P < 0.05$ or < 0.01) to these three variables in the first-half years. In the second-half years, four tested rice cultivars showed significant relationships with these three climatic variables, the content of amylose decreased with increasing temperatures above values of 19.5, 23.5 and 28.5℃ for MDMINAT, MDMAT and MDMAXAT, respectively.

The milled grain transparency of ‘TNG71’, ‘TK9’ and ‘KH145’ was not significantly correlated with MDMINAT, MDMAT and MDMAXAT in the first-half years, yet ‘TY4’ showed a significantly negative relationship with MDMAT and MDMAXAT. When MDMINAT, MDMAT and MDMAXAT raised above 20, 23.5 and 28.5℃, grain transparency decreased (Fig. 2). That is, the extent of whiteness (chalkiness) of milled grains increased with increasing temperatures during grain-filling period in all cultivars. The changes in head rice ratio of milled grains to MDMINAT, MDMAT and MDMAXAT showed that the negative relationship existed only in ‘TY4’ in the first-half years, while four cultivars all had a curvilinear relationship with these three variables in the second-half years (Fig. 3). Beyond mean temperatures of 20, 24 and 29.5℃ for MDMINAT, MDMAT and MDMAXAT, respectively, head rice ratio would decrease at harvest.

As shown in the aforementioned regression analyses, climatic variables MDMINAT, MDMAT and MDMAXAT calculated from grain-filling stage were related to yield and quality of all cultivars in the second-half years, but were found only applicable to ‘TY4’ in the first-half years. Such a result might be partially attributed the magnitude of temperature variations during grain-filling stage in the experimental period. Data showed that temperatures varied in ranges of 1.2–1.4℃ , 0.9–1.1℃ , 1.0–1.2℃ and 1.2℃ in the first half and increased to 1.6–2.4℃ , 1.5–2.1℃ , 1.8–2.7℃ and 1.7–2.5℃ in the second half for ‘TNG71’ , ‘TK9’ and ‘KH145’(data not shown), respectively, so that a larger temperature regime in the second-half years was given to a higher correlation than that of the first-half years. Moreover, the self-evident results also suggested that cultivar ‘TY4’ bred from cooler northern region was more sensitive to warm temperature and hence, its grain quality was more negative affected by high temperatures.

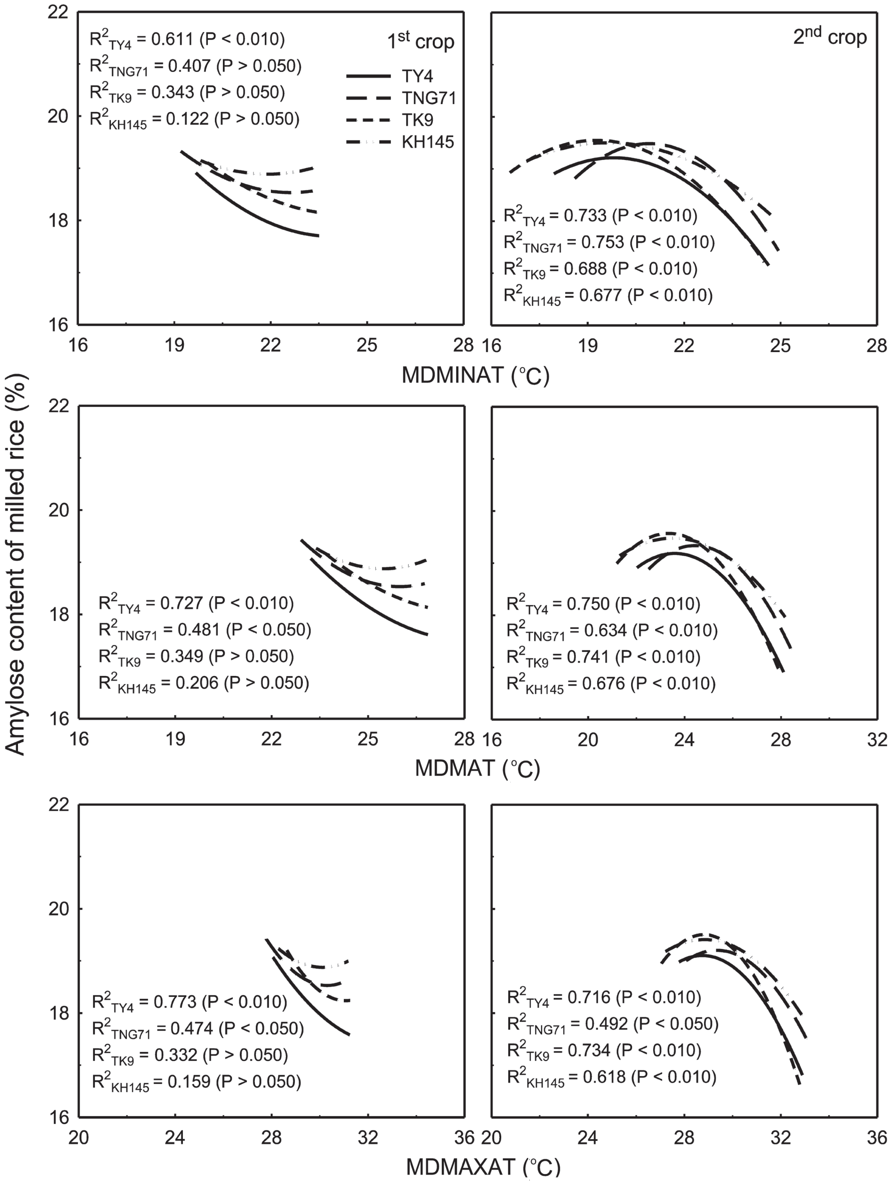

Fig. 1. Changes in amylose of milled rice to the mean values of daily minimum air temperature (MDMINAT), daily mean air temperature (MDMAT), and daily maximum air temperature (MDMAXAT) calculated from heading to harvest in the first and second crops of four rice cultivars ('TY4', 'TNG71', 'TK9', and 'KH145') grown in the cropping seasons of 2008–2010.

The distinct climatic conditions manifest in either the first or the second half of year and thus dictate a two-crop system of rice cultivation as well the differences in yield and quality of rice harvested in different crops in Taiwan (Chu & Lu 1984; Liu *et al*. 1984; Lu *et al*. 2008). As to the relationships of grain yield with MDMINAT, MDMAT and MDMAXAT of grain-filling stage, a quadratic function was fitted (Fig. 4). The relationship was significant for 'TNG71', 'TK9' and 'KH145', but not 'TY4', in the first-half years. By pooling all data col-

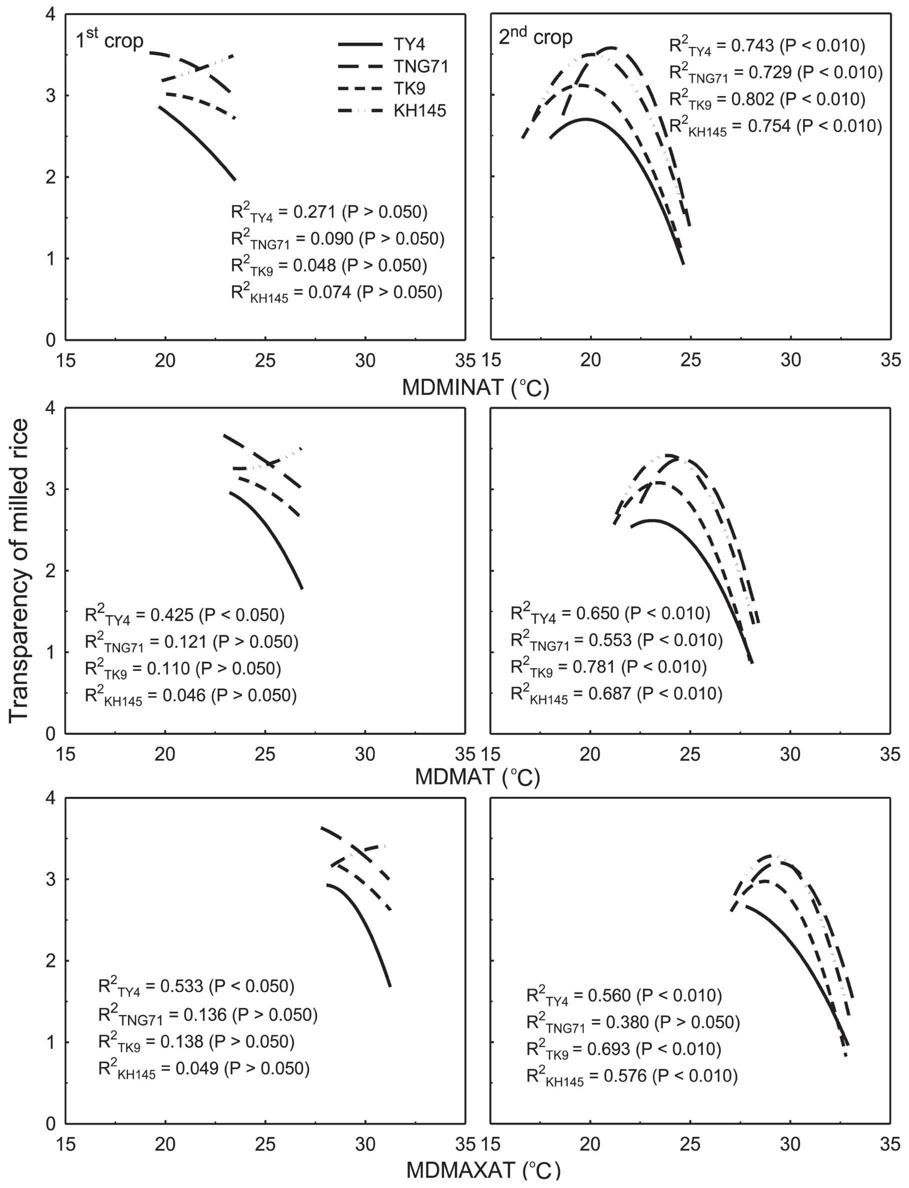

Fig. 2. Changes in transparency of milled rice to the mean values of daily minimum air temperature (MDMINAT), daily mean air temperature (MDMAT), and daily maximum air temperature (MDMAXAT) calculated from heading to harvest in the first and second crops of four rice cultivars (‘TY4’, ‘TNG71’, ‘TK9’, and ‘KH145’) grown in the cropping seasons of 2008–2010.

lected from all cultivars, the maximum yields achieved near 23, 26.5 and 31℃ for MDMINAT, MDMAT and MDMAXAT, respectively. A 2℃ increase for MDMINAT, MDMAT and MDMAXAT would cause respective 20, 18 and 16% yield decrease, implying a greater harmful effect of nighttime temperature (i.e., MDMINAT). In the second-half years, significant relationship was found in ‘TY4’ and ‘KH145’, but not in ‘TNG71’ and ‘TK9’, with MDMINAT. Again, the convex curve patterns of pooled data indicated a better temperature range for

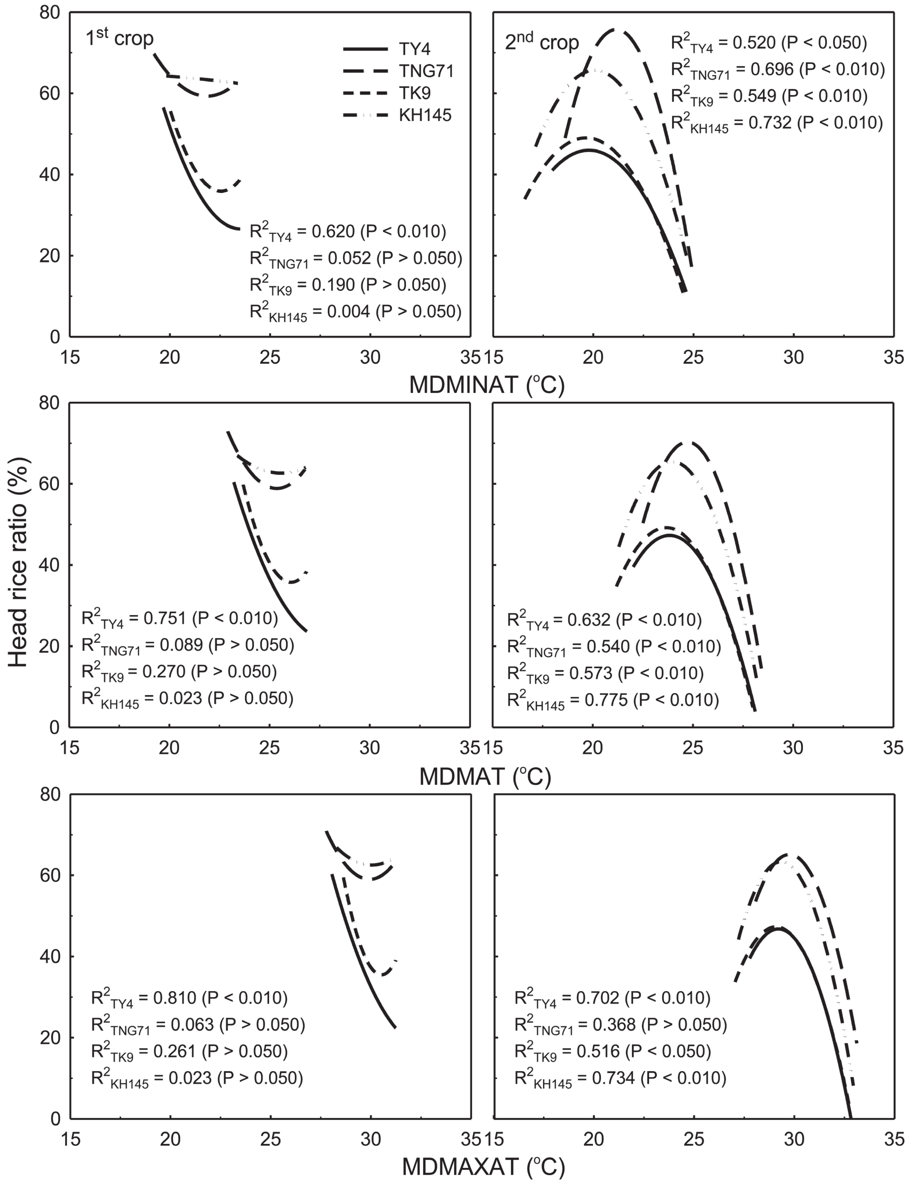

Fig. 3. Changes in head rice ratio to the mean values of daily minimum air temperature (MDMINAT), daily mean air temperature (MDMAT), and daily maximum air temperature (MDMAXAT) calculated from heading to harvest in the first and second crops of four rice cultivars (‘TY4’, ‘TNG71’, ‘TK9’, and ‘KH145’) grown in the cropping seasons of 2008–2010.

grain yield that centered near 20, 24 and 29℃ for MDMINAT, MDMAT and MDMAXAT, respectively. Temperature increases by 2℃ would result in 6, 9 and 7% drop of yield, an extent less than those of the first-half years.

Summarized from field investigations, Lur *et al.* (2006) pointed out that averaged values of daily minimum air temperature, daily mean air temperature and daily maximum air temperature lower than 22, 26 and 30℃ with daily irradiance higher than 13 MJ m^{-2} d^{-1} during grain-filling stage are a ‘safe and secure

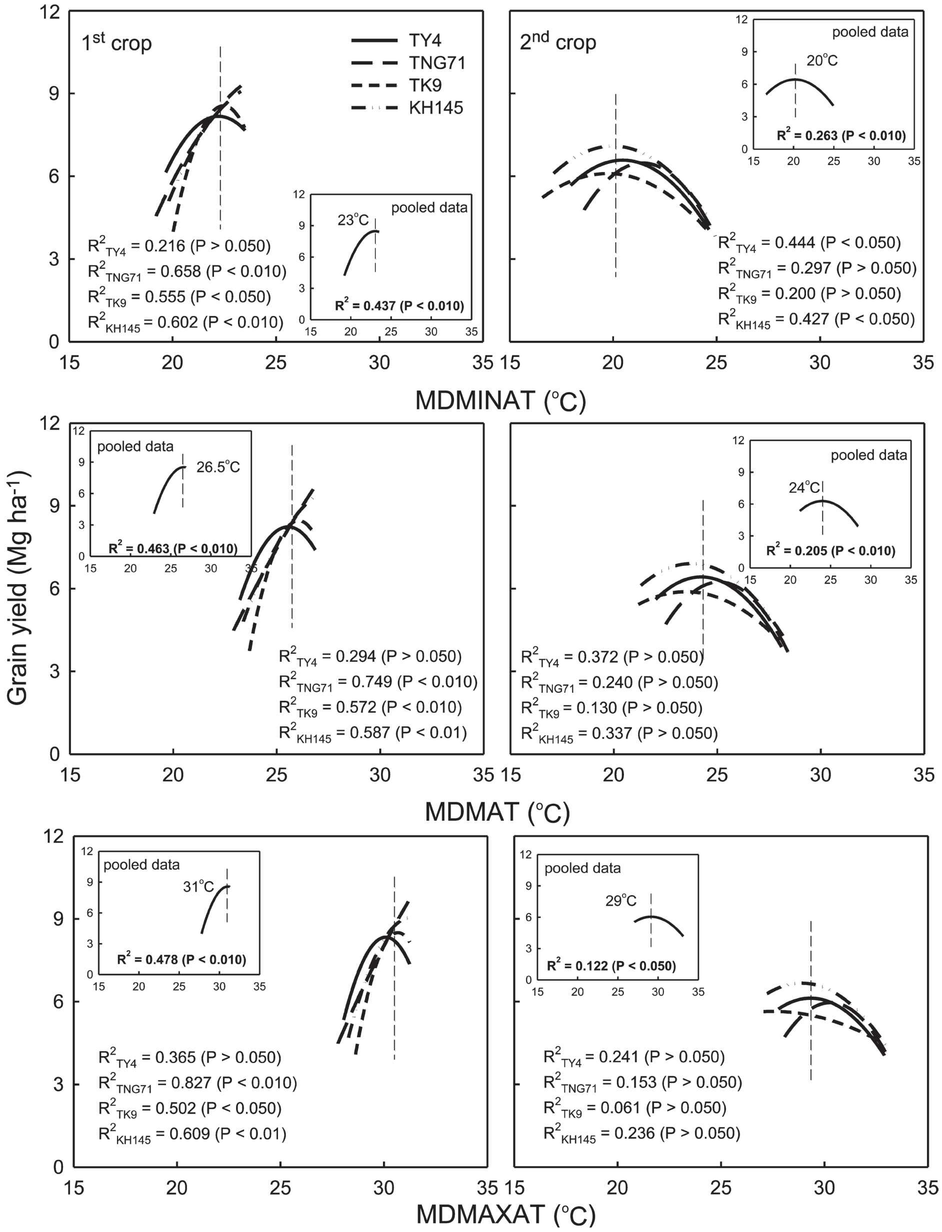

Fig. 4. Changes in grain yield of rice to the mean values of daily minimum air temperature (MDMINAT), daily mean air temperature (MDMAT), and daily maximum air temperature (MDMAXAT) calculated from heading to harvest in the first and second crops of four rice cultivars ('TY4', 'TNG71', 'TK9', and 'KH145') grown in the cropping seasons of 2008–2010.

environment' for quality rice production in Taiwan, in which conditions production of good appearance with low chalkiness grains can be high expected. This study further drew the outlines of a 'maximal yield environment' where MDMINAT, MDMAT and MDMAXAT of grain-filling stage near 23, 26.5 and 31℃ in the first-half year and 20, 24 and 29℃ in the second-half year, respectively, were the most suitable conditions for the selected rice culti-

vars to attain their maximum yield potential. Consequently, 5% variance for MDMINAT, MDMAT and MDMAXAT were computed as 22.4–23.6℃, 25.9–27.2℃ and 30.2–31.8℃ in the first-half year and 19.5–20.5℃, 23.4–24.6℃ and 28.3–29.7℃ in the second-half year to obtain 95–100% of their respective maximum yields. In other words, by adjusting planting seasons or selecting culture locations that can acquire such temperature ranges for respective cultivars, high yield of rice will be more reliable.

Both radiation and temperature are important to yield and quality of a crop. In the present research, grain yield was affected by MDNTD of grain-filling stage in the cropping seasons of the first-half years (Fig. 5), but not in the case of quality traits. However, amylose and transparency of milled grains, head rice ratio, and grain yield were significantly correlated with MDNTD in a positive curvilinear fashion in the second-half years. The increasing value of MDNTD would enhance quality traits and grain yield. The climatic variable MDIR was not significantly correlated with quality traits and grain yield in the first-half years, but influenced quality traits in the second-half years (Fig. 6). Quality traits improved with the increase of MDIR, reached the plateau, and then decreased thereafter. Nevertheless, the effects of integrated climatic factors on yield and quality of rice grains are issues worth further study and clarify, particularly to cultivars that bred from varied regions with diversified genetic contexts and environmental conditions.

Many studies reported that solar radiation and air temperature and even variation of nighttime temperature may also crucial for yield performance (Tsai *et al*. 1984; Morison 1992; Kukla & Karl 1993; Easterling *et al*. 1997; Peng *et al*. 2004; Islam &; Lee *et al*. 2009; Ho *et al*. 2012). Results from this study not only illustrated these facts but also demonstrated yield and quality of rice were fluctuated a greater extent in the second-half year than in the first-half year in Taiwan. Weather data collected in the experimental period proved a larger temperature and radiation variation in the cropping seasons of the second-half years (data not shown), which provide an unstable or even disturbed environment for growth and development of rice crops. As pointed out by Liu *et al*. (1984) that climatic factors will affect morphology and extent of expression of agronomic characters, and such phenomena vary in varieties, crops, locations and their interactions.

The prospects for future climate change and warming trend are substantial and are seemingly unavoidable and evitable based on the 2007 IPCC Fourth Assessment Report (IPCC 2007). As an example, results of this study indicated that 6–20% yield reduction would occur by 2℃ temperature increase in grain-filling stage of rice growth. To ameliorate the negative impacts on rice production and food security as a whole, strategic research plans are necessary on not just temperature but other climatic factors as well. With that, sufficient food production is feasible and applicable to meet perspectives of socio-economic consequences yielding from climate change and global warming. In addition to breeding approach for enhancing tolerance of crops to adverse environments and farming practices for improving crops adaptability (Sun *et al*. 2004; Lur *et al*. 2006; Lu *et al*. 2008), adjusting planting seasons and selecting culture locations are also proper strategies to adapt to abnormal climatic conditions in a changing climate.

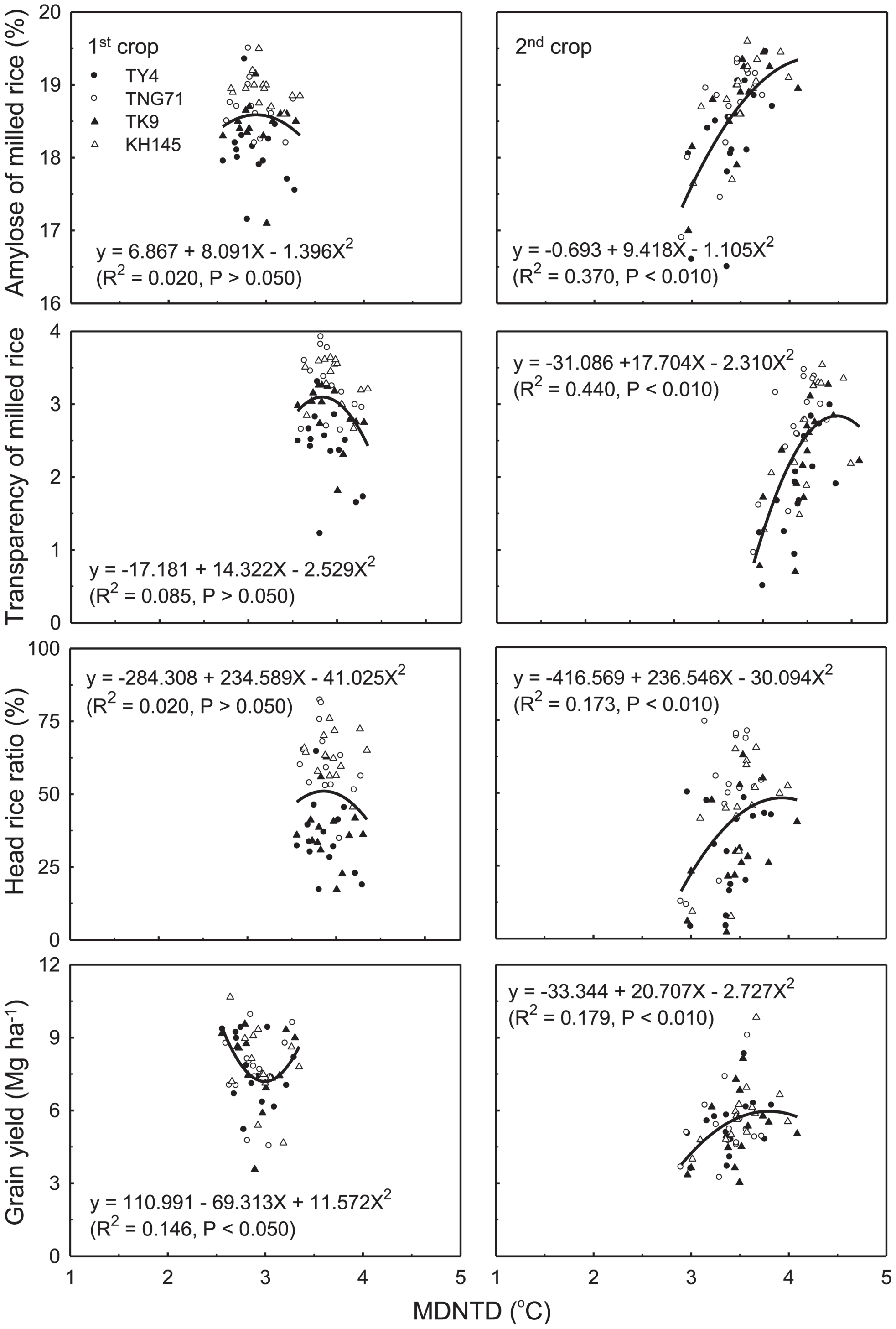

Fig. 5. Changes in amylose and transparency of milled rice, head rice ratio and grain yield to the mean values of day-night temperature difference (MDNTD) calculated from heading to harvest in the first and second crops of four rice cultivars ('TY4', 'TNG71', 'TK9', and 'KH145') grown in the cropping seasons of 2008–2010.

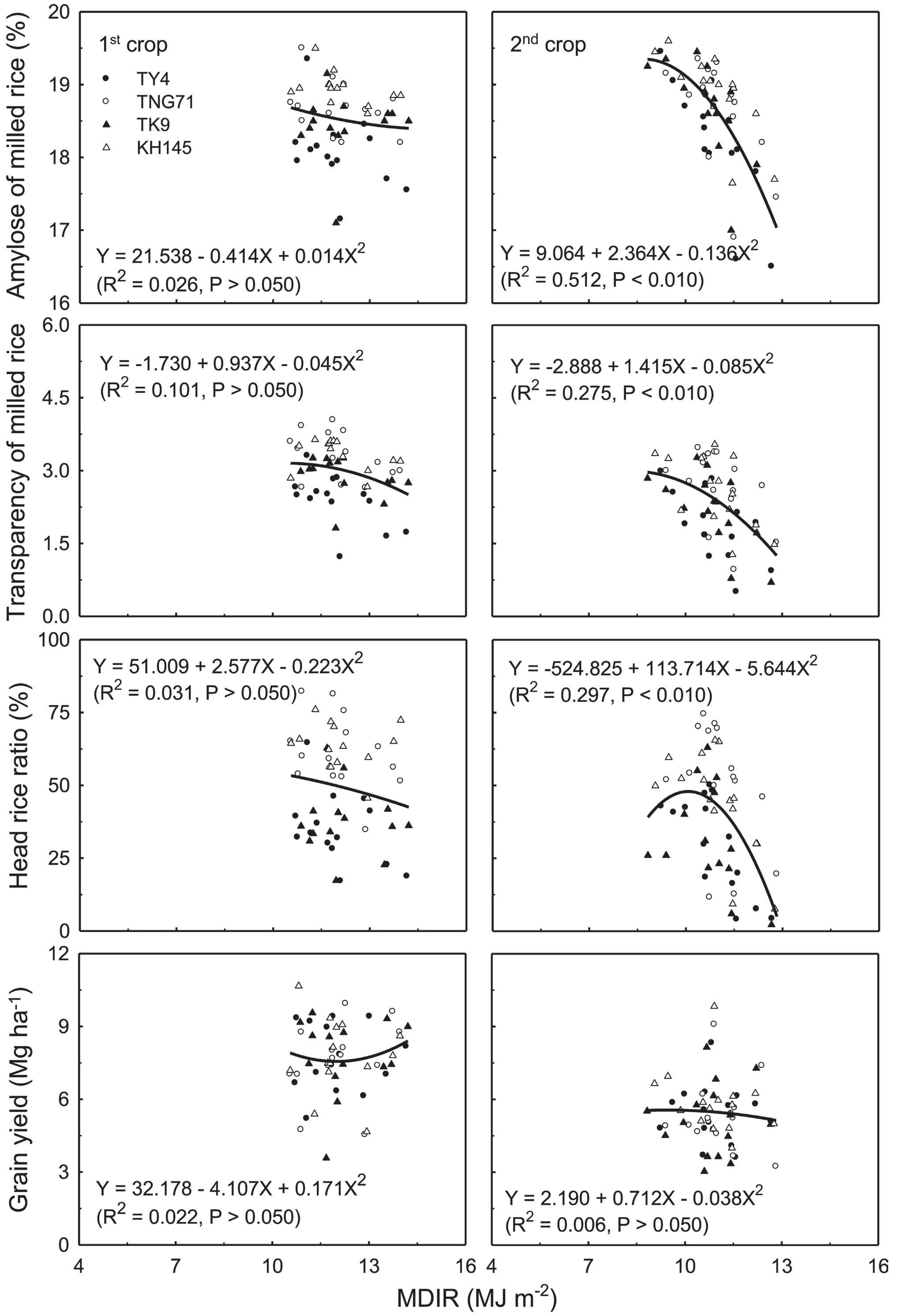

Fig. 6. Changes in amylose and transparency of milled rice, head rice ratio and grain yield to the mean values of daily irradiance (MDIR) calculated from heading to harvest in the first and second crops of four rice cultivars ('TY4', 'TNG71', 'TK9', and 'KH145') grown in the cropping seasons of 2008–2010.

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穀粒充實期間氣象因子對台灣不同地區選育稻種之米質與產量影響

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摘要

何佳勳、楊純明、蕭巧玲、賴明信。2013。穀粒充實期間氣象因子對台灣不同地區選育稻種之米質與產量影響。台灣農業研究 62(4):321–339。

由抽穗至收穫之穀粒充實期間氣象環境變化將影響稻米品質與產量，惟對選育自不同氣象環境的稻種將存在品種間差異性，探討與釐清這些差別效應將有助於建立各別地區性策略，以有效因應氣候變遷並穩定地區稻米供給而維護糧食安全。本研究於 2008–2010 年在行政院農業委員會農業試驗所農場 (台中市霧峰區) 進行三年期間上、下半年稻作之節氣栽植，三年總計 29 個栽植期。每個栽植期皆以 4 個良質稻種為試材，由北往南包括 '桃園 4 號' ('TY4')、'台農 71 號' ('TNG71')、'台稉 9 號' ('TK9') 及 '高雄 145 號' ('KH145') 等。試驗期間收集試區一級農業氣象測站之氣象測值，計算各稻種各栽植期自抽穗至收穫的穀粒充實期所量測的日均溫、日最高溫、日最低溫、日夜溫差、日照時數與日射量等六項氣象因子測值之平均值 (即 MDMAT、MDMAXAT、MDMINAT、MDNTD、MDSH、MDIR)，以分析六個氣象變因對不同稻種米質性狀與產量之差異影響。由回歸分析顯示，4 個良質稻種米質性狀受到穀粒充實期溫度變因 (MDMINAT、MDMAT 及 MDMAXAT) 影響，升溫將造成白米直鏈性澱粉、透明度及完整米率逐漸下降趨勢，惟上半年以 'TY4' 達顯著水準，下半年則 4 個稻種皆呈現顯著二次曲線關係。又產量與溫度變因之間為二次凸形曲線關係，各稻種有其產量表現之最佳平均值。總合 4 稻種資料，歸納出上半年栽植期之穀粒充實期最佳 MDMINAT 為 23℃、MDMAT 為 26.5℃、MDMAXAT 為 31℃時可獲得最大產量，而下半年則分別為 MDMINAT 20℃、MDMAT 24℃、MDMAXAT 29℃。另發現下半年各栽植期米質性狀與 MDNTD、MDIR 均呈現極顯著二次曲線關係，而穀粒產量則與 MDNTD 呈現極顯著二次回歸相關。綜此顯見不同稻種於穀粒充實期間，氣象環境的改變將影響米粒化學組成、物理特性及產量，而各米質性狀各有其不同之主要影響氣象變因與權重，且溫度上升超過最佳門檻值之後將下降。因此，為穩定稻米品質與產量，勢必篩檢合適栽植地區或栽植期以調適栽植期的氣象環境變化及未來變遷趨勢，尤其在氣候快速變遷情境下更需彈性因應。

關鍵詞：穀粒充實期間、氣象變因、不同地區選育稻種、米質、產量。

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利用塑酯薄膜法觀察甜瓜白粉病菌分生孢子的發芽與菌絲生長

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摘要

黃晉興、蘇俊峰。2013。利用塑酯薄膜法觀察甜瓜白粉病菌分生孢子的發芽與菌絲生長。台灣農業研究 62(4):340–350。

利用水瓊脂、醋酸纖維 (acetyl cellulose) 玻片與塑酯薄膜法 (plastic membrane method) 於顯微鏡下，皆可觀察到甜瓜白粉病菌 (*Podosphaera xanthii*) 分生孢子發芽，但是僅利用塑酯薄膜法才可觀察到白粉病菌的菌絲生長。將甜瓜白粉病菌分生孢子接種在葉圓片 (leaf disc) 上培養 48 h 後，利用塑酯薄膜法可觀察得到白粉病菌最適發芽溫度介於 20–28℃，發芽率介於 40.7–48.3% 之間，而最適菌絲生長溫度介於 20–26℃之間，菌絲產生率介於 30.7–33.1% 之間，其菌絲生長長度則介於 179.0–228.0 μm 之間。當甜瓜白粉病菌 (*P. xanthii race* 1) 分生孢子接種於感病甜瓜品種 'Iran H' 與 'Védrantais' 等之葉圓片 48 h 後，分生孢子發芽率、菌絲產生率與菌絲生長長度分別為 51.3–58.8%、41.3–53.5% 與 196.0–212.4 μm，皆高於接種於抗病甜瓜品種 'PMR-45'、'PI-124112' 與 'MR-1'，其分別為 12.5–15.4%、0–0.8% 與 0–82.1 μm，此結果與直接將 Px-001 供試菌株分生孢子接種於盆栽植株所得結果相同。又將 2 個 race 1 菌株 (Px-001 與 Px-274) 與 2 個 race 5 菌株 (Px-359 與 Px-3604) 分別接種在 'Iran H'、'PMR-45' 與 'MR-1' 品種葉圓片上，供試菌株在感病甜瓜品種的菌絲產生率在 29.0–54.0% 之間，而在抗病甜瓜品種的菌絲產生率在 0–6.8% 之間，具有顯著差異 ($P < 0.05$)。由結果顯示，將甜瓜白粉病菌分生孢子接種在甜瓜葉圓片上，再以塑酯薄膜法檢測分生孢子的菌絲產生率可做為甜瓜品種對白粉病菌之抗感性測定。

關鍵詞：塑酯薄膜法、甜瓜、白粉病菌、孢子發芽、菌絲生長。

前言

白粉病 (powdery mildew) 是甜瓜重要之地上部病害之一，此病害主要影響作物光合作用、降低瓜果風味、品質與產量 (Sitterly 1978; Tsay & Tung 1994; Zitter 1996)。引起該病害之病原菌主要包括 *Podosphaera xanthii* (Castag.) Braun & N. Shish [syn. *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll; S. *fusca* (Fr.) Blumer emend. Braun]、*Golovinomyces cichoracearum* (D. C.) Huleta (syn. *Erysiphe cichoracearum* DC ex. Mecat) 及 *Leveillula taurica* (Lev.) Arnaud (Pitrat *et al*. 1998; Braun *et al*. 2001; Jahn *et al*. 2002)。其中以 *P. xanthii* 較為常見，一般多發生於熱帶、亞熱帶地區以及溫室栽培園內，而 *G. cichoracearum* 多發生於溫帶地區，*L. taurica* 則較少見 (Křístková *et al*. 2007)。在台灣，目前僅有 *P. xanthii* 被鑑定為甜瓜白粉病的病原菌 (Tsay & Tung 1994; Huang *et al*. 2002)。

甜瓜白粉病菌 (*P. xanthii*) 有許多生理小種 (races) 的區分 (Mohamed *et al*. 1995; Pitrat *et al*. 1998; McCreight 2006)，在台灣目前有 race 1 與 race 5 等 2 個生理小種，race 1 可感染甜瓜鑑別品種 'Iran H' 與 'Védrantais'，並

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出現病徵，但不會在 ‘PMR-45’、‘Edisto-47’ 與 ‘WMR-29’ 等鑑別品種造成病徵，而 race 5 可在甜瓜鑑別品種 ‘Iran H’、‘ Védrantais’、‘PMR-45’、‘Edisto-47’ 與 ‘WMR-29’ 造成病徵，而甜瓜鑑別品種 ‘PI-124112’ 與 ‘MR-1’ 對上述 2 個生理小種皆呈抗病反應 (Mohamed *et al*. 1995; Pitrat *et al*. 1998; McCreight 2006; Huang & Wang 2007)。上述這些甜瓜鑑別品種對白粉病菌的抗感病反應，皆可利用葉圓片接種法或全株接種法判定 (Cohen 1993; Huang *et al*. 2002)。

甜瓜白粉病菌為一種表生之絕對寄生性真菌，只能利用寄主活體的養分，無法於人工培養基生長與繁殖。其分生孢子於寄主植物表面發芽，爾後產生附著器 (appresoria)、侵入釘 (infection peg)，侵入植物組織內以吸器 (haustoria) 吸取葉細胞的養分，使菌絲得以於葉表蔓延、生長形成菌落，產生分生孢子並進行傳播 (Sitterly 1978)。若要觀察孢子於寄主作物葉表面的行為，不易以解剖顯微鏡直接觀察，需先將葉片以化學藥劑透化與染色，才能在光學顯微鏡 (Kuzuya 2006; Romero *et al*. 2008) 或掃描式電子顯微鏡下觀察 (Romero *et al*. 2008)。此過程中卻不易將未發芽的孢子固定在葉表上，另外此過程繁瑣，不易進行大量標本的觀察；若將孢子吹佈於水瓊脂培養基或醋酸纖維玻片 (acetyl cellulose) 上，雖可觀察得到所有接種的孢子，且部分孢子會有發芽管產生，但是發芽後的孢子卻無法繼續生長產生菌絲 (Tsay & Tung 1994)。為了觀察孢子發芽後於葉表面的生態行為，必須研發更適用的方法。

可撕噴霧式薄膜 (airbrush shrink wrap) 其主要成分為醋酸丁酯 (butyl acetate) 與聚氯乙稀 (poly vinyl chloride; PVC)，為一種液態塑膠產物。以噴霧方式將之噴佈於物品表面，待乾燥後可形成一層薄而透明且具有彈性之保護膜，達到防塵的效果。因此，本研究擬利用可撕噴霧式薄膜液體，將其噴佈於甜瓜葉片上，嘗試將葉表面上的白粉病菌分生孢子黏著，將薄膜撕下後置於顯微鏡下染色，觀察孢子於葉表面發芽與菌絲生長，該方法稱為塑酯薄膜法 (plastic membrane method)。

材料與方法

供試植物與葉圓片之製備

將 ‘Iran H’、‘Védrantais’、‘PMR-45’、‘PI-414723’ 與 ‘MR-1’ 等 5 個甜瓜品種，種植於盛有泥炭土 (Bio-Mix Potting Substrate, Netherlands) 之 5 吋塑膠盆內，置於 28℃/22℃ (day/night) 12 h 光照之生長箱中栽培。待供試植物苗齡 3–4 wk 時，取苗齡 3 wk 植株上半部第 3、4 葉片或苗齡 4 wk 第 4–7 葉之新展開葉片，以自來水洗淨，再經消毒水 (於 200 mL 1% 次氯酸鈉加入數滴 Tween-20 界面活性劑攪拌均勻) 表面消毒 1–2 min 後，以無菌水漂洗 3 次，再以乾淨衛生紙吸乾葉表面之游離水。已經高溫滅菌後之金屬圓柱打孔器切取直徑 15 mm 葉圓片 (面積 1.77 cm^2)，置於裝有二張濾紙之塑膠培養皿內 (直徑 6 或 9 cm)，該培養皿內再添加適量含有 50 μL L^{-1} tetracyclin 之 M-solution (水溶液含 10,000 μL L^{-1} 的 mannitol 與 30 μL L^{-1} 的 benzimidazole)，使濾紙完全濕潤，並有游離水產生，用以維持葉圓片的活力 (Epinet *et al*. 1993; Bardin *et al*. 1997; Huang *et al*. 2002)。

甜瓜白粉病菌接種源準備與接種方法

本文使用之供試菌 (*P. xanthii*) 繁殖於葉片上，經由乾燥後保存於液態氮中 (Huang *et al*. 2009)，菌株計有 Px-001、Px-274、Px-359 與 Px-360，皆分離自台中市霧峰區發生白粉病之甜瓜葉片，前 2 個菌株為 race 1，後 2 個菌株為 race 5。將供試菌株移植於上述葉圓片培養皿內 (甜瓜品種為 ‘Iran H’)，於 12 h 光照的 20℃ 定溫箱中培養 12–16 d，使白粉病菌長滿整個葉圓片作為接種源。接種白粉病菌時，於中空塑膠圓椎管 (底管口直徑 12 cm，上管口直徑 4 cm，高 30 cm) 底管口處放置一含葉圓片之培養皿，於上管口處以氣流將供試菌株分生孢子吹落於置於底管口處的供試葉圓片，亦或可接種於苗齡 3 wk 的栽培盆甜瓜幼苗葉片上。接種後，使被接種的葉圓片上達 100–300 spores cm^{-2} (Huang *et al*. 2002)，培

養皿加蓋後，並以石臘膜密封，並移置於每日 12 h 光照的 24℃/18℃ (day/night) 定溫箱中，10 d 後記錄發病面積佔葉片之百分比，視爲發病度 (disease severity)。

利用水瓊脂、醋酸纖維玻片與塑酯薄膜檢測甜瓜白粉病菌孢子發芽與菌絲生長

將醋酸纖維 (acetyl cellulose, Sigma) 溶解於丙酮 (W：W = 1：100) 再塗抹於載玻片上，以製成醋酸纖維玻片，放置在裝有濕潤濾紙的 9 cm 培養皿中；又配置 2% 水瓊脂培養基 [water agar (WA), Difco, USA] 與以 'Iran H' 品種甜瓜植株製備葉圓片。利用上述中空塑膠圓椎管接種方式，將供試菌株 (Px-001) 的分生孢子，分別接種於醋酸纖維玻片、2% WA 與葉圓片上 (100–300 spores cm^{-2})，培養皿以石臘膜密封，每處理 4 重複，並移置於每日 12 h 光照的 24℃生長箱中培養 48 h。接種後的 2% WA 與醋酸纖維玻片直接於光學顯微鏡下觀察孢子發芽與菌絲生長的情形。而以葉圓片接種法處理之葉圓片，以可撕噴霧式塑酯薄膜噴劑 (可撕噴霧式保潔膜，巧幫手公司，台灣) 噴佈葉圓片表面，待蔭乾後撕下薄膜，以酸性品紅染劑 (acid-fusion) 進行染色，於光學顯微鏡下觀察孢子發芽與菌絲生長的情形 (圖 1)。由於分生孢子產生發芽管後，並不一定

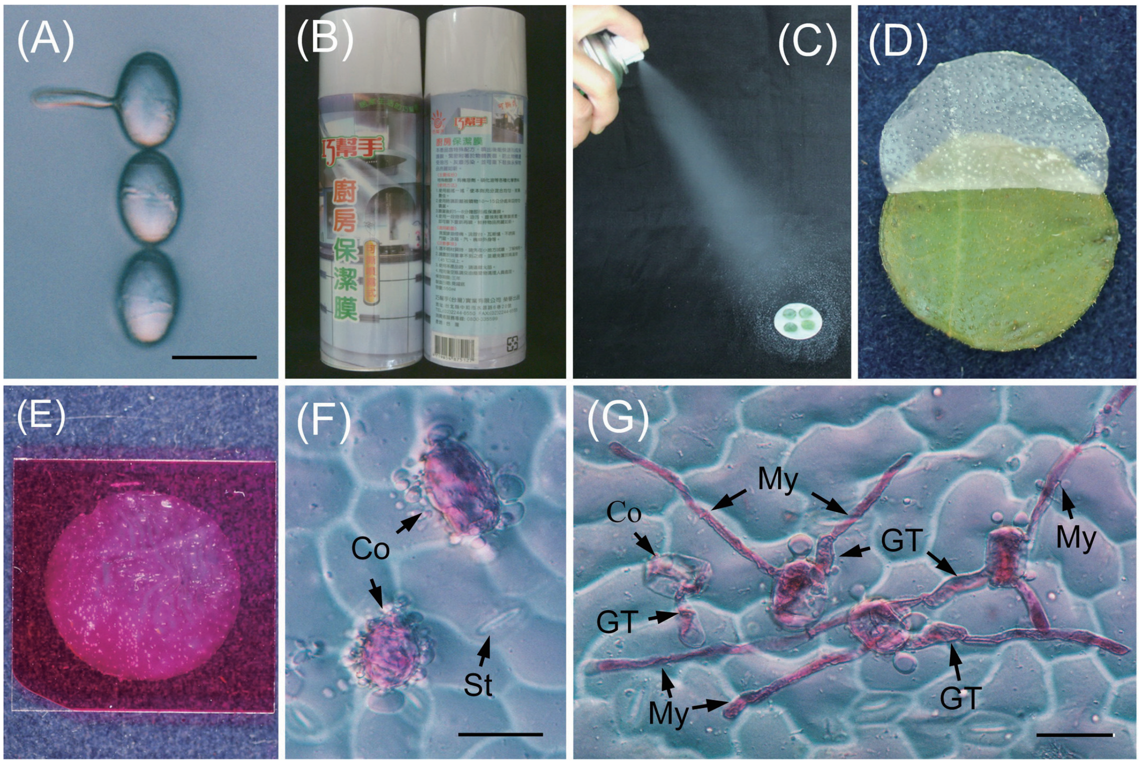

圖 1. 白粉病菌 *Podosphaera xanthii* 分生孢子之發芽與菌絲生長觀察。分生孢子於水瓊脂發芽 (A)；利用液態塑酯 (B) 噴霧於附著有甜瓜白粉病菌孢子的甜瓜葉圓片上 (C)，撕下塑酯薄膜 (D) 經過酸性品紅染劑染色後 (E)，於顯微鏡下可觀察黏附於塑酯薄膜的白粉病菌分生孢子的發芽與菌絲生長 (F、G)。Co：分生孢子；ST：氣孔；GT：發芽管；My：菌絲。

Fig. 1. Conidial germination and mycelial growth *Podosphaera xanthii*. Germination of conidia on water agar (A). Liquid plastic resin (B) spraying on a leaf disc of melon inoculated with conidia of *P. xanthii* (C) and the plastic membrane was removed (D) and stained with acid fusion (E). Conidia, germinated conidia and mycelia from germinated conidia which adhered on plastic membrane (F, G) were examined under compound microscope. Co: conidia; St: Stomata; GT: germ tube; My: mycelia. Bar = 25 µm.

會產生菌絲 (圖 1G)，故觀察時，每重複觀察 100 個孢子，並計算發芽率 (germination rate, %) 與菌絲產生率 (hyphal development rate, %)，再量取最長菌絲長度，每重複量取 10–20 條菌絲。

溫度對甜瓜白粉病菌分生孢子發芽與菌絲生長的影響

以 'Iran H' 品種製備葉圓片接種供試菌株 Px-001 (100–200 spores cm^{-2})，接種後移置於 4、8、12、16、20、24、26、28、30、32 與 36℃的定溫箱中 (光照 12 h d^{-1})，分別於接種後第 0、3、6、12、24 與 48 h，於葉圓片上噴佈可撕噴霧式薄膜，待蔭乾後撕下薄膜，經酸性品紅染劑染色，於光學顯微鏡下觀察、計算孢子發芽率與菌絲產生率，並逢機取 10 顆有已長出菌絲的孢子，量取菌絲長度，每處理 4 皿 (重複)，每皿 4 片葉圓片。

品種對甜瓜白粉病菌分生孢子發芽與菌絲生長的影響

以 'Iran H'、'Védrantais'、'PMR-45'、'PI-414112' 與 'MR-1' 等 5 個甜瓜品種製備葉圓片，接種供試菌株 Px-001 達 100–200 spores cm^{-2}，供試葉圓片於 24℃生長箱中培養 48 h。另一處理，則將上述品種種植在盛有泥炭土之 5 吋塑膠盆中，待株齡 3 wk 時，將供試菌株 Px-001 的分生孢子接種於的第 3–4 片展開葉上。接種後，於 24℃生長箱中培養 48 h (光照 12 h d^{-1})，以打孔器切取下直徑 15 mm 葉圓片。於試驗所得之葉圓片上噴佈可撕噴霧式薄膜，待蔭乾後撕下薄膜，經以酸性品紅染劑染色，於光學顯微鏡下觀察、計算孢子發芽率與菌絲產生率，並逢機取 10 顆已長出菌絲的孢子，量取菌絲長度，每處理 4 皿 (重複) ，每皿 4 片葉圓片。又於接種後第 10 d，計算發病度。

不同白粉病菌與甜瓜品種對分生孢子發芽與菌絲生長的影響

以 'Iran H'、'PMR-45' 與 'MR-1' 等 3 個甜瓜品種製備葉圓片，接種甜瓜白粉病菌 *P. xanthii* 供試菌株 Px-001 (race 1)、Px-274 (race 1)、Px-359 (race 5) 與 Px-360 (race 5) 達 100–200 spores cm^{-2}。供試葉圓片於 24℃生長箱中培養 48 h 後，於葉圓片上噴佈可撕噴霧式薄膜，待蔭乾後撕下薄膜，經以酸性品紅染劑染色，於光學顯微鏡下觀察、計算孢子發芽率與菌絲產生率，並逢機取 10 顆已長出菌絲的孢子，量取菌絲長度，每處理 4 皿 (重複)，每皿 4 片葉圓片。又於接種後第 10 d，於解剖顯微鏡下將白粉菌產生孢子佔葉圓片之面積比率視爲發病度，計算各處理之發病度。

結果

塑酯薄膜法之孢子回收率

將接種白粉病菌孢子的甜瓜葉圓片 48 h 後，以塑酯薄膜法黏附孢子與菌絲 (圖 1)，計算 4 次接種於葉圓片的孢子量分別爲 147、190、233 與 251 spores cm^{-2}，再計算經塑酯薄膜黏附的孢子量分別爲 150、186、226 與 238 spores cm^{-2}，則以塑酯薄膜可回收接種的分生孢子達 97.9%。

利用水瓊脂、醋酸纖維玻片與塑酯薄膜觀察白粉病菌孢子發芽與菌絲生長

將接種白粉病菌孢子的 2% WA 培養基與醋酸纖維玻片 48 h 後，直接於光學顯微鏡下可直接觀察到白粉病菌的孢子發芽 (圖 1A)，但是無法觀察到有菌絲生長。而將接種白粉病菌的葉圓片表面噴佈可撕噴霧式塑酯薄膜 (圖 1B、1C)，待蔭乾後撕下附著孢子的薄膜 (圖 1D)，再以酸性品紅染劑染色 (圖 1E) 後，於光學顯微鏡直接鏡檢白粉病孢子 (圖 1F)，並可觀察到已發芽的孢子與菌絲生長 (圖 1G)。供試菌株 Px-001 分生孢子接種於水瓊脂、醋酸纖維玻片與葉圓片 48 h 後，其發芽率分別爲 5.6、47.6 與 49.2%，而在葉圓片上的已發芽孢子有菌絲生長出，菌絲產生率爲 41.2%，其菌絲長度爲 187.4 μm (表 1)。

溫度對白粉病菌孢子發芽與菌絲生長的影響

以 'Iran H' 品種製備葉圓片接種供試菌株 Px-001，置於 4–36℃定溫箱中，3 h 後，16–32℃處理者已經開始有少數分生孢子發芽。孢

表 1. 白粉病菌 *Podosphaera xanthii* 之分生孢子於水瓊脂、醋酸纖維玻片、Iran H 甜瓜葉圓片上的發芽與菌絲生長。

Table 1. Conidial germination and mycelial growth of *Podosphaera xanthii* on 2% water agar, 2% cellulose acetate and leaf discs of the melon variety Iran H[z].

Substrate	Conidial germination (%)[y]	Hyphal development rate (%)[x]	Hyphal length (μm 48 h^{-1})[w]
2% water agar	5.6 b[v]	0.0 b	0.0 b
2% cellulose acetate	47.6 a	0.0 b	0.0 b
Leaf discs of Iran H	49.2 a	41.2 a	187.4 a

[z] The data was recorded 48 h after inoculation at 24℃.
[y] Percentage of conidia produced germ tube.
[x] Percentage of germinated conidia developed hyphae from germ tubes.
[w] Mean radial length of mycelia from germ tubes.
[v] Means within each column followed by the same letter are not significantly different at $P < 0.05$ according to the Fisher's least significance difference (LSD) test.

子發芽率隨處理時間增加而增加，在處理後 48 h，以 20、24、26 與 28℃處理的發芽率較高，分別為 40.7、48.3、47.8 與 41.3%。隨溫度降低，發芽率亦隨之減少，16℃以下處理，孢子發芽率皆低於 30%。同樣的，隨溫度增加，發芽率亦隨之減少，30℃以上處理的發芽率皆低於 17%，36℃則完全不發芽 (圖 2A)。而已發芽的分生孢子，於接種處理 12 h 後已可觀察到有菌絲生長出，到接種後 48 h 的菌絲產生率在 20、24 與 26℃之處理者為較高，分別為 33.1、31.6 與 30.7%；12、16 與 28℃之處理次之，分別為 9.8、19.9 與 15.7%；30℃之處理為 3.9%，而 4、8、32 與 36℃處理者，在測試時間內皆無菌絲生長出 (圖 2B)。在量取接種後 48 h 的菌絲生長長度，20、24 與 26℃處理者最高，分別為 179.0、228.0 與 180.4 μm；28 與 30℃之處理次之，分別為 150.0 與 103.7 μm；12 與 16℃之處理則分別為 47.8 與 89.3 μm；4 與 8℃在 48 h 則不見菌絲生長 (圖 2C)。

品種對甜瓜白粉病菌分生孢子發芽與菌絲生長的影響

以 'Iran H'、'Védrantais'、'PMR-45'、'PI-414112' 與 'MR-1' 等 5 個甜瓜品種製備葉圓片，接種供試菌株 Px-001。接種 48 h 後，供試菌株的分生孢子在 'Iran H' 與 'Védrantais' 葉圓片上發芽率分別為 55.1% 與 51.3%，兩者無顯著差異 ($P < 0.05$)，但是在 'PMR-45'、'PI-414112' 與 'MR-1' 葉圓片上的發芽率介於 12.5–15.4% 之間，三者間無顯著差異 ($P < 0.05$)，但是其與 'Iran H' 與 'Védrantais' 所得之發芽率具有顯著差異 ($P < 0.05$)。而供試菌株在 'Iran H' 與 'Védrantais' 葉圓片的菌絲產生率分別為 41.3% 與 44.3%，但在 'PMR-45'、'PI-414112' 與 'MR-1' 葉圓片上卻僅有 0–0.8%。已發芽的分生孢子在 'Iran H' 與 'Védrantais' 葉圓片的菌絲生長長度分別為 196.0 μm 與 204.5 μm，但在 'PMR-45'、'PI-124112' 與 'MR-1' 葉圓片上分別為 67.0、82.1 與 0 μm。進一步計算發病度，在 'Iran H' 與 'Védrantais' 葉圓片上已達的 100%，但在 'PMR-45'、'PI-414112' 與 'MR-1' 葉圓片上則皆為 0 (表 2)。

另一處理，則將 'Iran H'、'Védrantais'、'PMR-45'、'PI-414112' 與 'MR-1' 等 5 個甜瓜品種種植在盛有泥炭土之 5 吋塑膠盆中，待株齡 3 wk 時，再接種供試菌株 Px-001 的分生孢子。結果在接種 48 h 後，供試菌株分生孢子在 'Iran H' 與 'Védrantais' 葉片上的發芽率分別為 56.3% 與 47.6%，兩者無顯著差異 ($P < 0.05$)，而在 'PMR-45'、'PI-414112' 與 'MR-1' 處理者的發芽率介於 21.3–29.4%，雖然三個品種處理間無顯著差異 ($P < 0.05$)，但與 'Iran H' 與 'Védrantais' 處理的發芽率具有顯著差異 ($P < 0.05$)。菌絲產生率在 'Iran H' 與 'Védrantais' 處理分別為 50.8% 與 43.8%，與 'PMR-45'、'PI-414112' 與 'MR-1' 處理間具有顯著差

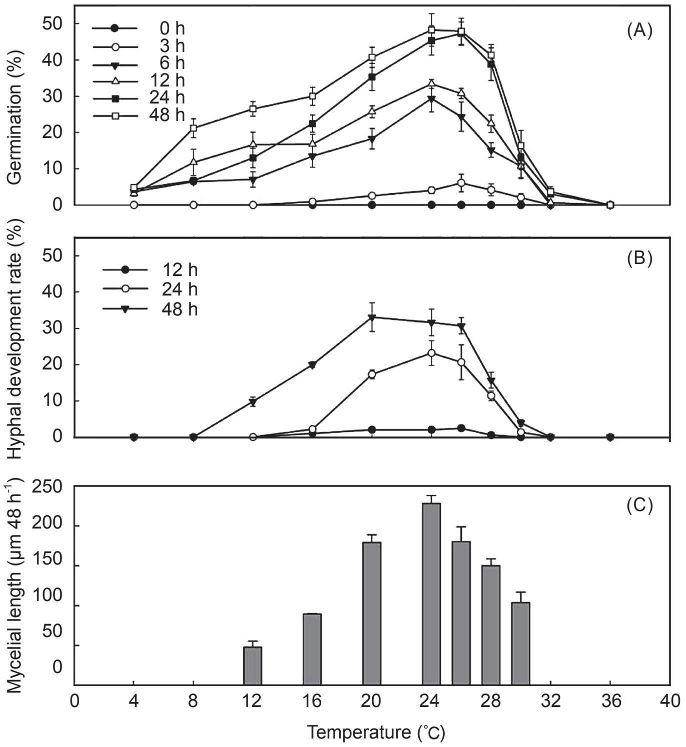

圖 2. 溫度對白粉病菌 *Podosphaera xanthii* 分生孢子於甜瓜 Iran H 葉圓片上的發芽 (A)、菌絲產生 (B) 與菌絲長度 (C) 之影響。

Fig 2. Effect of temperature on germination of conidia (A), production of hyphae from germ tubes (B) and length of mycelia from gerinated conidia (C) of *Podosphaera xanthii* inoculated on leaf discs of the melon variety Iran H. Data were collected from inoculated samples after incubation for 0, 3, 6, 12, 24 and 48 h (A); 12, 24 and 48 h (B); and 48 h (C).

表 2. 白粉病菌 *Podosphaera xanthii* (race 1) 之分生孢子於甜瓜抗感病品種葉圓片上的發芽與菌絲生長。

Table 2. Conidial germination and mycelial growth of *Podosphaera xanthii* (race 1) on leaf-discs of resistant and susceptible varieties of melon[z].

Variety	Conidial germination (%)[y]	Hyphal development rate (%)[x]	Hyphal length (μm 48 h^{-1})[w]	Disease severity (%)[v]	Disease reaction
Iran H	55.1 a [u]	41.3 a	196.0 a	100.0 a	Susceptible
Védrantais	51.3 a	44.3 a	204.5 a	100.0 a	Susceptible
PMR-45	15.4 b	0.5 b	67.0 b	0.0 b	Resistant
PI-124112	12.5 b	0.8 b	82.1 b	0.0 b	Resistant
MR-1	15.0 b	0.0 b	0.0 c	0.0 b	Resistant

[z] The data was recorded 48 h after inoculation at 24℃.
[y] Percentage of conidia produced germ tube.
[x] Percentage of germinated conidia developed hyphae from germ tubes .
[w] Mean radial length of mycelia from germ tubes.
[v] Percentage of leaf-disc area of melon showing symptoms of powdery mildew.
[u] Means within each column followed by the same letter are not significantly different at $P < 0.05$ according to the Fisher's least significance difference (LSD) test.

異，其菌絲產生率介於 0–0.3%。菌絲生長長度在 'Iran H' 與 'Védrantais' 處理分別為 174.2 μm 與 181.9 μm，與 'PMR-45'、'PI-414112' 與 'MR-1' 處理間具有顯著差異 ($P < 0.05$)，其菌絲生長長度分別為 63.1、0 與 0 μm。計算發病度，'Iran H' 與 'Védrantais' 處理的發病面積

達 95.0 與 97.2%，但是在 ‘PMR-45’、‘PI-414112’ 與 ‘MR-1’ 處理卻僅有 0–0.2% (表 3)。

不同白粉病菌與甜瓜品種對分生孢子發芽與菌絲生長的影響

甜瓜白粉病菌 *P. xanthii* Px-001 (race 1)、Px-274 (race 1)、Px-359 (race 5) 與 Px-360 (race 5) 等 4 個菌株在 ‘Iran H’ 品種的葉圓片上的孢子發芽率介於 50.0–61.0%，菌絲產生率介於 37.3–54.0%，而菌絲生長長度介於 160.0–229.0 μm，接種後發病度可達 96.7–100.0% 之間。在 ‘PMR-45’ 品種上，供試菌株 Px-001 與 Px-274 的孢子發芽率分別為 17.8–31.5%，與供試菌株 Px-359 與 Px-360 的發芽率分別為 46.0 與 49.8%，具有顯著差異 ($P < 0.05$)。而 Px-001 與 Px-274 在 ‘PMR-45’ 品種上的菌絲產生率分別為 0.3 與 2.5%，與 Px-359 與 Px-360 的菌絲產生率 29.0 與 32.5%，具有顯著差異 ($P < 0.05$)。Px-001 與 Px-274 在 ‘PMR-45’ 品種上的菌絲生長長度分別為 21.1 與 87.8 μm，與 Px-359 與 Px-360 的菌絲生長長度 144.4 與 220.8 μm，具有顯著差異 ($P < 0.05$)。計算發病面積，則 Px-001 與 Px-274 在 ‘PMR-45’ 品種上的發病面積僅為 0 與 5%，與 Px-359 與 Px-360 的發病面積 70 與 73.7%，具有顯著差異 ($P < 0.05$)。在 ‘MR-1’ 品種上，供試的 4 株菌株孢子發芽率分別為 19.3、22.3、42.3 與 27.3%，以供試菌株 Px-359 處理的孢子發芽率較高，菌絲產生率分別為 2.0、0、1.3 與 6.8%，彼此間無顯著差異 ($P < 0.05$)，菌絲生長長度分別為 29.3、0、80.4 與 171.7 μm，供試菌株 Px-001 與 Px-274 所得結果與供試菌株 Px-359 與 Px-360 所得結果具有顯著差異 ($P < 0.05$)。計算發病面積後，發現 4 株供試菌株在 ‘MR-1’ 品種上的發病面積介於 0–2.7%，彼此間無顯著差異 ($P < 0.05$) (表 4)。

討論

由於甜瓜白粉病菌為絕對寄生菌，故需要在寄主活體上才能獲得生長所需的養分 (Sitterly 1978; Alexopoulos *et al*. 1996)，然而本研究的結果顯示，在無寄主的環境下 (如在高濕度的水瓊脂平板與醋酸纖維玻片上)，甜瓜白粉病菌分生孢子仍可發芽，但在水瓊脂平板上發芽率低，而在醋酸纖維玻片上則發芽率提高，顯示在粗糙接觸面之介質則可能有利於發芽。然而孢子在此 2 介質之發芽率皆較葉片上低，顯示除了高濕度與粗糙接觸面之外，寄主尚有其他因子可促進孢子發芽。在水瓊脂平板與醋酸纖維玻片上，分生孢子雖能發芽產生發芽管，但卻無法長出菌絲，可見分生孢子發芽時外界的養分供應不是絕對需要，但要進一步產生菌絲則需自寄主獲得所需養分。

表 3. 白粉病菌 *Podosphaera xanthii* (race 1) 於盆栽甜瓜抗感病品種葉片上的孢子發芽與菌絲生長。

Table 3. Conidial germination,mycelial growth and disease sevirity of *Podosphaera xanthii* (race 1) inoculated on leaves of susceptible and resistant melon varieties grown in pots[z].

Variety	Conidial germination (%)[y]	Hyphal development rate[x]	Hyphal length (μm 48 h^{-1})[w]	Disease severity (%)[v]	Disease reaction
Iran H	56.3 a [u]	50.8 a	174.2 a	97.2 a	Susceptible
Védrantais	47.6 a	43.8 a	181.9 a	95.0 a	Susceptible
PMR-45	29.4 b	0.3 b	63.1 b	0.2 b	Resistant
PI-124112	21.3 b	0.0 b	0.0 c	0.0 b	Resistant
MR-1	21.9 b	0.0 b	0.0 c	0.0 b	Resistant

[z] The data was recorded 48 h after inoculation at 24℃.
[y] Percentage of conidia produced germ tube.
[x] Percentage of germinated conidia developed hyphae from germ tubes.
[w] Mean radial length of mycelia from germ tubes.
[v] Percentage of leaf-disc area of melon showing symptoms of powdery mildew.
[u] Means within each column followed by the same letter are not significantly different at $P < 0.05$ according to the Fisher’s least significance difference (LSD) test.

表 4. 以塑酯薄膜法測定不同白粉病菌株於甜瓜抗感病品種葉圓片上的孢子發芽與菌絲生長。

Table 4. Conidial germination, mycelial growth and disease severity of different *Podosphaera xanthii* isolates inoculated on leaf-discs of resistant and susceptible melon varieties[z].

Variety	Isolate[y]	Conidial germination (%)[x]	Hyphal development rate (%)[w]	Hyphal length (μm 48 h^{-1})[v]	Disease severity (%)[u]	Disease reaction
Iran H	Px-001	61.0 ab[t]	41.0 b	191.8 abc	100.0 a	Susceptible
	Px-274	49.8 bc	38.3 bc	193.2 abc	100.0 a	Susceptible
	Px-359	50.0 bc	37.3 bc	160.3 cd	96.7 a	Susceptible
	Px-360	58.5 ab	54.0 a	229.0 a	96.7 a	Susceptible
PMR-45	Px-001	17.8 e	2.5 d	87.8 e	0.0 c	Resistant
	Px-274	31.5 de	0.3 d	21.1 f	5.0 c	Resistant
	Px-359	49.8 bc	29.0 c	144.4 d	73.7 b	Susceptible
	Px-360	46.0 c	32.5 bc	220.8 ab	70.0 b	Susceptible
MR-1	Px-001	19.3 e	2.0 d	29.3 f	0.0 c	Resistant
	Px-274	22.3 e	0.0 d	0.0 f	0.0 c	Resistant
	Px-359	42.0 cd	1.3 d	80.4 e	0.0 c	Resistant
	Px-360	27.3 e	6.8 d	171.7 cd	2.7 c	Resistant

[z] The data was recorded 48 h after inoculation at 24℃.
[y] Isolates Px-001 and Px-274 are race 1 and isolates Px-359 and Px-360 are race 5.
[x] Percentage of conidia produced germ tube.
[w] Percentage of germinated conidia developed hyphae from germ tubes.
[v] Mean radial length of mycelia from germ tubes.
[u] Percentage of leaf-disc area of melon showing symptoms of powdery mildew.
[t] Means within each column followed by the same letter are not significantly different at $P < 0.05$ according to the Fisher's least significance difference (LSD) test.

本研究結果顯示，接種於甜瓜葉片上的甜瓜白粉病菌分生孢子發芽適溫是 20–28℃，而菌絲生長之最適溫是 20–26℃。28℃發芽率雖可達 41.3%，但菌絲產生率卻僅有 15.7%，且菌絲長度較短。依據 Huang *et al*. (2006) 的報告，甜瓜白粉病病勢發展最快的溫度是 20–24℃，可見分生孢子發芽之適溫，不見得是病害發生之適溫，而菌絲生長的適溫與病勢發展適溫則較為相近。

過去研究在白粉病抗、感病品種篩選上，常利用自然感染 (Schnathorst 1965; Aust 1986)、人工接種在盆栽植株 (Cohen & Cohen 1986) 或葉圓片 (Kaur & Jhooty 1986; Cohen 1993; Epinet *et al*. 1993; Mohamed *et al*. 1995; Bardin *et al*. 1997)。一般接種後呈現抗、感病反應的判讀時間至少需要 10 d，而由本研究利用塑酯薄膜分析的結果顯示，48 h 即可利用顯微鏡觀察得到孢子的發芽率、菌絲產生率與菌絲生長長度，這些皆與植株表現抗、感病反應有關。發芽率、菌絲產生率高或菌絲生長長度長者，多呈現感病反應，反之則為抗病反應。而且葉圓片接種的反應與盆栽接種的反應相同，顯示在孢子發芽的階段，寄主與病原菌之間即有交互作用，一直持續到菌絲生長。然而在本研究中，有白粉病菌菌系的孢子發芽率或菌絲生長長度在感病與抗病甜瓜品種無顯著差異，如表 4 中的 Px-359 (race 5) 菌株在感病甜瓜品種 ‘PMR-45’ 或抗病品種 ‘MR-1’ 之分生孢子發芽率分別為 49.8% 或 42.0%，二者無顯著差異；Px-360 (race 5) 菌株在抗病品種 ‘MR-1’ 的 48 h 菌絲生長長度達 171.7 μm，已和一般感病反應的菌絲生長長度差不多，所以無法以孢子發芽率與菌絲生長長度做為抗感病反應的早期判斷。然而菌絲產生率與甜瓜品種抗感病反應相符，接種在感病品種之供試菌株菌絲產生率大於 29.0%，在抗病品種則低於 6.8%。故可在接種甜瓜白粉病菌分生孢子 48 h 後，利用塑酯薄膜法於顯微鏡下計算孢子菌絲產生率，作為甜瓜對白粉病菌抗感性的早期判斷。

在利用塑酯薄膜來檢測孢子在葉片上發芽

與菌絲生長的最大優點是方便且快速，不像葉肉透化染色或是掃描式電子顯微鏡的標本處理可能會讓未發芽孢子從葉表面上掉落。筆者曾使用石臘包埋，但耗時費工，且石臘不夠透明而不利觀察。黏附在塑酯薄膜的白粉病菌孢子與菌絲很容易在光學顯微鏡下觀測，不過此方法也有其限制，在接種超過 48 h 以後，菌絲生長密佈的情形下，則不易在顯微鏡下觀察塑酯薄膜上的孢子發芽與否，而附著器 (appresorium) 與吸器 (haustorium) 也不易用此方法觀察。塑酯薄膜法不僅可適用於觀察甜瓜白粉病菌分生孢子在甜瓜葉表上的發芽與菌絲生長，未來應可應用在寄生在寄主表面的其他白粉病菌、其他絕對寄生菌 (如某些銹病菌) 或其他表生真菌。

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Observation of Conidia Germination and Mycelial Growth of *Podosphaera xanthii* on Melon Leaf by Plastic Membrane Method

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Abstract

Huang, J. H. and J. F. Su. 2013. Observation of conidia germination and mycelial growth of *Podosphaera xanthii* on melon leaf by plastic membrane method. J. Taiwan Agric. Res. 62(4):340–350.

A study revealed that conidial germination of *Podosphaera xanthii* was observed under light microscope when they were inoculated on water agar, acetyl cellulose or leaf discs of melon and retrieved by a plastic membrane method. However, mycelial growth was observed only by the plastic membrane method. When conidia of *P. xanthii* (race 1) were inoculated on melon leaf-discs (Iran H variety), incubated for 48 h at series temperatures, and prepared specimens with the plastic membrane method for observation under compound microscope, the range of optimum temperature for conidial germination was 20–28℃ with the germination rate of 40.7–48.3%. The range of optimum temperature for hyphal development from germinated conidia and mycelial growth was 20–26℃, with the hyphal development rate of 30.7–33.1% and the hyphal length of 179.0–228.0 μm. When conidia of *P. xanthii* (race 1) were inoculated on leaf-discs of the susceptible melon varieties, 'Iran H' and 'Védrantais', and incubated at 24℃ for 48 h, the range of conidial germination rate, hyphal development rate and hyphal length were 51.3–58.8%, 41.3–53.5% and 196.0–212.4 μm, respectively. They were significantly ($P < 0.05$) higher than those (12.5–15.4%, 0–0.8%, and 0–82.1 μm) of the pathogen inoculated on the resistant melon varieties, 'PMR-45', 'PI-124112' and 'MR-1'. When the conidia of P. xanthii (race 1) were inoculated on leaves of the tested melon varieties grown in pots, the conidial germination, hyphal development rate and hyphal length were similar to the results of conidia inoculation on the leaf-discs. The conidia of race 1 strains (Px-001 and Px-274) and race 5 strains (Px-359 and Px-360) of *P. xanthii* were respectively inoculated on leaf discs of the susceptible variety Iran H and the resistant varieties, 'PMR-45' and 'MR-l', the hyphal development rate (29.0–54.0%) on the susceptible melon varieties was significantly ($P < 0.05$) higher than that (0–6.8%) on the resistant varieties. These results suggest that the plastic membrane method for collecting samples from leaf discs or leaves of plants inoculated with conidia of *P. xanthii* may be a useful and practical method for effective rating of melon varieties for resistance to powdery mildew.

Key words: Plastic membrane method, Melon, *Podosphaera xanthii*, Conidial germination, Mycelial growth.

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溫度對檬果小黃薊馬 (*Scirtothrips dorsalis* Hood) 發育與繁殖之影響

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摘要

陳怡如、林鳳琪、邱一中、石憲宗。2013。溫度對檬果小黃薊馬 (*Scirtothrips dorsalis* Hood) 發育與繁殖之影響。台灣農業研究 62(4) :351–359。

小黃薊馬 (*Scirtothrips dorsalis* Hood) 為影響檬果 (*Mangifera indica* Linn) 產量與品質的關鍵害蟲。本研究以檬果葉片飼育小黃薊馬，於20、24、28及32℃之恆溫下觀察其生活史，分析溫度對其發育與繁殖之影響，作為未來研擬防治策略之參考。結果顯示，在4個溫度下未成熟期薊馬之存活率均達80%以上；由卵發育至成蟲所需時間依溫度升高而縮短，分別為21.5、14.8、12.1及9.8 d；雌蟲的平均壽命依序為26.3、17.4、19.8及13.2 d；每一雌蟲一生總產卵數依序為37.3、38.4、74.0及40.0粒。由溫度與發育速率之直線回歸方程式，估算小黃薊馬卵至成蟲之發育臨界低溫為9.6℃，發育總積溫為219.1日度 (degree-days)。以台南地區全年之有效積溫估計，小黃薊馬在檬果上年可發生約40世代，每年2–6月氣溫適合其生活發育與繁殖，也是其在檬果園發生高峰期。

關鍵詞：小黃薊馬、檬果、發育、產卵。

前言

檬果 (*Mangifera indica* Linn) 原產於熱帶，台灣栽培品種以愛文、在來種、金煌及凱特為主，種植面積達1萬6千公頃，主要產地在台南及屏東 (Anonymous 2011)。檬果上發生害蟲種類多達80種 (Wen & Wu 2010; Shih *et al*. 2013)，其中小黃薊馬為影響檬果產量與品質的關鍵害蟲 (Lin *et al*. 2010a)。

小黃薊馬 (*Scitothrips dorsalis* Hood, chilli thrips)，屬纓翅目薊馬科 (Thysanoptera: Thripidae)，未成熟期包含卵、幼蟲及蛹期，卵為腎形，小於0.2 mm，產於植物幼嫩組織中；一齡及二齡幼蟲均在葉面活動，前蛹及蛹為靜止期，不喜活動亦不取食。成蟲體型小，體長0.7–0.9 mm，體色淡黃。食性雜，已記錄寄主植物達40科110種以上，分布於亞洲、大洋洲及赤道周圍等國家 (Venette & Davis 2004; Wang & Hsu 2007; CABI 2010)。在台灣早年為害茶樹，又名茶黃薊馬，近年在台南玉井檬果園的蟲害監測顯示，每年1–2月間檬果萌花時小黃薊馬密度便急速上升 (Lin *et al*. 2010a, 2010b)，可為害新梢、花穗及幼果，造成新芽及葉片萎縮褐化，在花穗造成傷口間接發生萎凋，並直接取食果實造成果皮粗糙褐化之疤痕 (Lee & Wen 1982)，影響檬果產銷價格甚鉅。

小黃薊馬在田間環境條件適合時，族群密度增長非常迅速 (Lin *et al*. 2010a)，若無法掌握時機，適時防治，將引起嚴重危害造成經濟損失。昆蟲的族群增長受溫度與食物之影響最為明顯，故探討溫度及寄主植物對其存活、發

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育及繁殖影響，有助於害蟲的發生預測，以及在害蟲管理系統中決定防治與否及採行之技術。國內外關於小黃薊馬發育及繁殖研究報導甚少，往昔曾以茶樹及蓖麻 (Dev 1964; Patel *et al*. 2009)、珊瑚樹、溫州蜜柑 (Tatara 1994)、葡萄 (Shibao 1996)、菜豆、茄子、甜椒、南瓜、番茄及玫瑰等爲寄主植物 (Seal *et al*. 2010)，進行相關之研究分析。本研究以愛文檬果爲寄主植物飼育小黃薊馬，探討溫度與其發育繁殖的關係，藉以評估其在田間族群發生趨勢，作爲掌握田間防治適期及研擬防治策略之參考。

材料與方法

供試蟲源與植物

2010 年於台南市玉井區採集帶有小黃薊馬之檬果嫩葉，裝入透氣的夾鍊袋中，攜回實驗室內飼養，作爲觀察薊馬發育、存活、成蟲壽命與產卵試驗的供試蟲源。

採集農委會農業試驗所栽種之愛文檬果嫩葉，攜回實驗室以清水洗淨葉片，再以紙巾輕拭葉面水滴。先在解剖顯微鏡下鏡檢，若葉肉組織中，已經被產卵，則以蟲針挑除，確保葉片乾淨無蟲後，放入冰箱 (4℃) 冷藏保存，作爲後續薊馬生活史觀察之供試葉片。使用葉片時須再裁切成圓形 (diam. 28 mm)，放入杯底置有圓型濕潤紙片 (diam. 28 mm) 的平底玻璃管 (高 35 mm，內徑 30 mm) 中，供薊馬產卵及食物來源。

未成熟期之存活率與發育觀察

挑取供試蟲源中活動力強的小黃薊馬雌成蟲，移入有供試葉片玻璃管，以 2 層石蠟膜 (Parafilm® M) 封住玻璃管口，以微小蟲針在上方戳洞，減少水氣凝結於管壁。再以保鮮膜封口後並戳洞，分別置於設定 20、24、28 及 32℃ 四個恆溫 (± 1℃) 之梯度恆溫生長箱中 (L：D = 12：12)，任其產卵 24 h 後挑除雌成蟲，將帶有被產卵葉片之玻璃管分別置於不同恆溫下，觀察其生活史。

每日定時取出供試葉片觀察記錄卵之孵化情形，並以 3 mL 之滴管滴入 1 滴水於玻璃管中，維持管內紙巾濕潤，避免供試葉片乾枯而影響卵的孵化。待一齡幼蟲孵化後，用微毛筆將供試蟲挑至個別玻璃管內進行單隻飼養，隔日更換新鮮葉片，每日定時觀察記錄幼蟲個體之變化、存活等情形，直到發育爲成蟲，計算其雌雄性比。

成蟲壽命與繁殖

前項試驗之薊馬羽化變成成蟲後，進行配對飼養。當日羽化雌成蟲，挑取同一試驗溫度下飼養羽化之雄蟲進行配對，若當日雄蟲缺乏無法配對時，則由供試蟲源中挑取並配對。每日取出已產卵葉片，在解剖顯微鏡下觀察計算產卵數，並更換新鮮葉片供其每日產卵，直至成蟲自然死亡爲止。

統計分析

由不同恆溫下小黃薊馬卵至成蟲發育所需日數，以直線回歸方程式 $D(T) = a + bT$，求取發育速率與溫度之關係，其中 T 爲飼養小黃薊馬的溫度 (℃)；D 爲恆溫下薊馬的發育速率，爲發育所需日數之倒數。由估算所得回歸方程式，推算薊馬的發育總積溫 (thermal summation; $K = 1/b$) 及發育臨界低溫 (low temperature threshold; $T_0 = -a/b$)。

其中，發育總積溫 (K) 的標準誤差 (standard error) 由下列公式計算：

$$\frac{\mathrm{S.E.\ of\ b}}{b^2}$$

而發育臨界低溫 (T_0) 的標準誤差，則以下列公式計算：其中 $\bar{y}$ 是樣本平均，N 是樣本數量，S^2 是樣本的殘差平均平方和 (residual mean square)。

$$\frac{\bar{y}}{b}\sqrt{\frac{S^2}{N\bar{y}^2}+\left[\frac{\mathrm{S.E.\ of\ b}}{b}\right]^2}$$

結果

孵化率與存活率

小黃薊馬於 20、24、28 及 32℃ 之恆溫下，

卵孵化率依序為 86.4、94.8、100 及 99.3%。由卵發育至成蟲的累積死亡率在20及32℃時，兩者之累積死亡率均大於 15%，其餘溫度下所飼育者均低於 10% (表 1)。

未成熟期發育所需時間

在 20、24、28 及 32℃ 等 4 個恆溫下，小黃薊馬發育所需時間依序由低至高溫，卵期為 9.7、7.0、5.5 及 4.2 d；一齡幼蟲期為 2.8、2.1、1.6 及 1.3 d；二齡幼蟲期為 4.1、2.3、2.3 及 2.0 d；前蛹期為 1.3、1.1、0.8 及 0.6 d；蛹期為 3.5、2.3、1.9 及 1.7 d。由卵發育至成蟲平均所需日數，依序分別為 21.5、14.8、12.1 及 9.8 d，隨溫度增高而縮短 (表 2)，在未成熟蟲期中以卵發育為幼蟲所需時間最長。

成蟲壽命、性比及繁殖量

在 20、24、28 及 32℃ 恆溫下，小黃薊馬成蟲壽命與產卵數如表 3 所示。在各試驗溫度下，雌蟲壽命較雄蟲長，雌蟲的壽命依序為 26.3、17.4、19.8 及 13.2 d，雄蟲的壽命為 17.3、9.8、10.4 及 8 d。除飼養於 28℃ 者其壽命較飼育於 24℃ 者長，其餘雌雄成蟲壽命隨著溫度的升高而縮短。觀察雌蟲每日產卵數，由低至高溫依序為 1.4、2.0、3.8 及 2.8 粒；總產卵量為 37.3、38.4、74.0 及 40.0 粒，以飼育於 28℃ 時之雌成蟲之平均日產卵數和總產卵數最高。在 4 種溫度下，以飼育於 24℃ 及 28℃ 者產雌率較高，雌雄比分別為 2.7：1 及 2.57：1 (表 3)。

溫度與發育速率之關係

根據本試驗所得數據以直線回歸，分析溫度對檬果上小黃薊馬卵至成蟲發育速率之關係方程式為：$D(T) = -0.044 + 0.00456\ T$，$R^2 = 0.9972$，$P < 0.05$，推算由卵發育至成蟲之發育臨界低溫為 9.6℃ ± 0.2℃，其發育總積溫 (K) 為 219.1℃ 日度 ± 9.4 日度 (degree-days)。溫度與卵及各齡幼蟲及蛹之發育速率均呈直線關係 (圖 1)，估算卵期、一齡幼蟲期、二齡幼蟲期、前蛹期及蛹期之發育臨界低溫，依序為 10.9、9.8、5.9、11.8 及 7.7℃，其發育總積溫依序為 90.9、29.5、49.5、12.3 及 39.7 日度。

討論

本試驗觀察結果，小黃薊馬在檬果葉上

表 1. 小黃薊馬於不同恆溫下在檬果上之卵孵化率及幼期累積死亡率。

Table 1. Hatching rate and cumulative mortality of the immature stage of *Scirtothrips dorsalis* at various constant temperatures on mango.

				Cumulative mortality (%)			
Temp. (℃)	n	Hatching (%)	n	1st larva	2nd larva	Propupa	Pupa
20	162	86.4	88	4.5	10.2	1.1	19.3
24	173	94.8	85	0.0	3.5	4.7	7.1
28	91	100.0	65	0.0	1.5	7.7	9.2
32	147	99.3	96	1.0	5.2	8.3	16.7

表 2. 小黃薊馬於不同恆溫下在檬果上之幼期平均發育所需時間。

Table 2. Mean developmental times (days) of immature stages of *Scirtothrips dorsalis* at various constant temperatures on mango.

Temp. (℃)	Egg	1st larva	2nd larva	Propupa	Pupa	Egg-adult
20	9.7 ± 1.4[z]	2.8 ± 0.6	4.1 ± 1.2	1.3 ± 0.5	3.5 ± 0.6	21.5 ± 2.0
24	7.0 ± 0.4	2.1 ± 0.4	2.3 ± 0.7	1.1 ± 0.3	2.3 ± 0.5	14.8 ± 0.8
28	5.5 ± 0.7	1.6 ± 0.5	2.3 ± 0.9	0.8 ± 0.4	1.9 ± 0.3	12.1 ± 1.0
32	4.2 ± 0.6	1.3 ± 0.5	2.0 ± 0.8	0.6 ± 0.5	1.7 ± 0.5	9.8 ± 1.1

[z] Mean ± standard deviation.

表 3. 小黃薊馬於不同恆溫下在檬果上小黃薊馬之成蟲壽命、性比及繁殖率。

Table 3. Adult longevity, sex ratio, fecundity of *Scirtothrips dorsalis* at various constant temperatures on mango.

	Temp. (℃)			
Trait	20	24	28	32
Adult longevity (d)				
Female	26.3 ± 15.5[z]	17.4 ± 7.5	19.8 ± 8.0	13.2 ± 6.1
Male	17.3 ± 6.5	9.8 ± 4.1	10.4 ± 3.6	8.0 ± 4.1
No. eggs of female				
Daily	1.4 ± 0.8	2.0 ± 0.9	3.8 ± 1.5	2.8 ± 1.5
Total	37.3 ± 26.3	38.4 ± 25.0	74.0 ± 33.4	40.0 ± 28.0
Sex ratio (♀ : ♂)	1.36	2.7	2.57	1.29

[z] Mean ± standard deviation.

孵化與存活，在 4 個觀察恆溫下，20℃時卵之孵化率 86.4%，在 24℃時卵之孵化率提升為 94.8%，至 28℃時，所觀察之卵全部皆孵化為幼蟲，但在 32℃時則略降低為 99.3%，顯示孵化率在 20–28℃時隨著溫度升高而增加。幼蟲發育至成蟲之存活情形，在 20 或 32℃之累積死亡率均達 15% 以上，顯示溫度過高或過低均不利其存活。根據文獻報導，Shibao (1996) 指出在 16、20、25、30℃等恆溫下，以葡萄為寄主飼育小黃薊馬，其孵化率為 42.6–50% 均低於本試驗各恆溫下之 86.4–100%；Tatara (1994) 指出以珊瑚樹 (sweet viburnum) 為飼育寄主，在 33℃及 34.5℃時，約 80% 以上卵可孵化，但無法完成發育，當溫度達 36℃，所觀察的卵均無法孵化。本試驗在 32℃時，小黃薊馬卵仍有 99.3% 之孵化率，高於前人研究飼育葡萄及珊瑚樹之結果。由此也顯示，台南地區檬果上之小黃薊馬族群已適應高於 30℃之氣候環境。本研究觀察溫度 32℃時，其未成熟期累積死亡率高於飼育於 24℃及 28℃之薊馬，顯示小黃薊馬在超過 32℃的高溫下存活率較差。Shibao (1996) 在 16–30℃四個恆溫下，以葡萄飼育之小黃薊馬之未成熟期之死亡率均達 50%，相較之下以檬果飼育者有較高之存活率。

在 20–32℃時，小黃薊馬未成熟期各階段發育日數，均隨溫度增高而縮短，在飼育溫度範圍內，由卵發育至成蟲平均需 9.8–21.5 d。其他文獻報導在不同溫度以不同植物飼育小黃薊馬 (Dev 1964; Tatara 1994; Shibao 1996; Seal *et al*. 2010)，其未成熟期發育日數，在 20℃時飼育以葡萄需 26.6 d；在 24–25℃時飼育以葡萄、茶、珊瑚樹葉片，依序需 18.4、17.2、18.6 d；在 26℃時，飼育以番椒、玫瑰、茄子、南瓜、菜豆及番茄等，依序需 17.8、18.4、18.9、20.2、20.3 及 20.6 d； 在 27–28℃時飼育以蓖麻需 16.9 d；以上前人研究結果與本試驗在 20、24 及 28℃恆溫下觀察結果相較，在相近溫度下，以檬果葉飼育小黃薊馬，其未成熟期所需發育日數均短於飼育以其他寄主植物者，且存活率較高。以昆蟲生理與生態之理論而言，寄主植物之營養越適合植物之生長，其存活率高、所需發育時間越短、及繁殖力較強，顯示檬果較其他供試植物更適合小黃薊馬生長發育。

本研究在同一恆溫下飼育小黃薊馬，其 5 個生活期的長短以卵最長，在 20–32℃的 4 個觀察恆溫下，依序為 9.7、7.0、5.5 及 4.2 d；其發育所需日數佔總發育期 (21.5、14.8、12.1 及 9.8 d) 之 43–47%。

由於薊馬產卵於植物組織內，卵相對於蟲體暴露於生活環境中的幼蟲、蛹及成蟲受到更多保護，作者曾測試數種對小黃薊馬幼蟲殺蟲效果達 80% 以上之殺蟲劑 (Chiu *et al*. 2010a)，對葉組織中小黃薊馬卵的殺蟲效果，結果顯示 90% 以上供試卵均可孵化 (作者未發表資料)。為提高小黃薊馬之防治率，建議在化學防治策略上 (Lin *et al*. 2010a; Chiu *et al*.

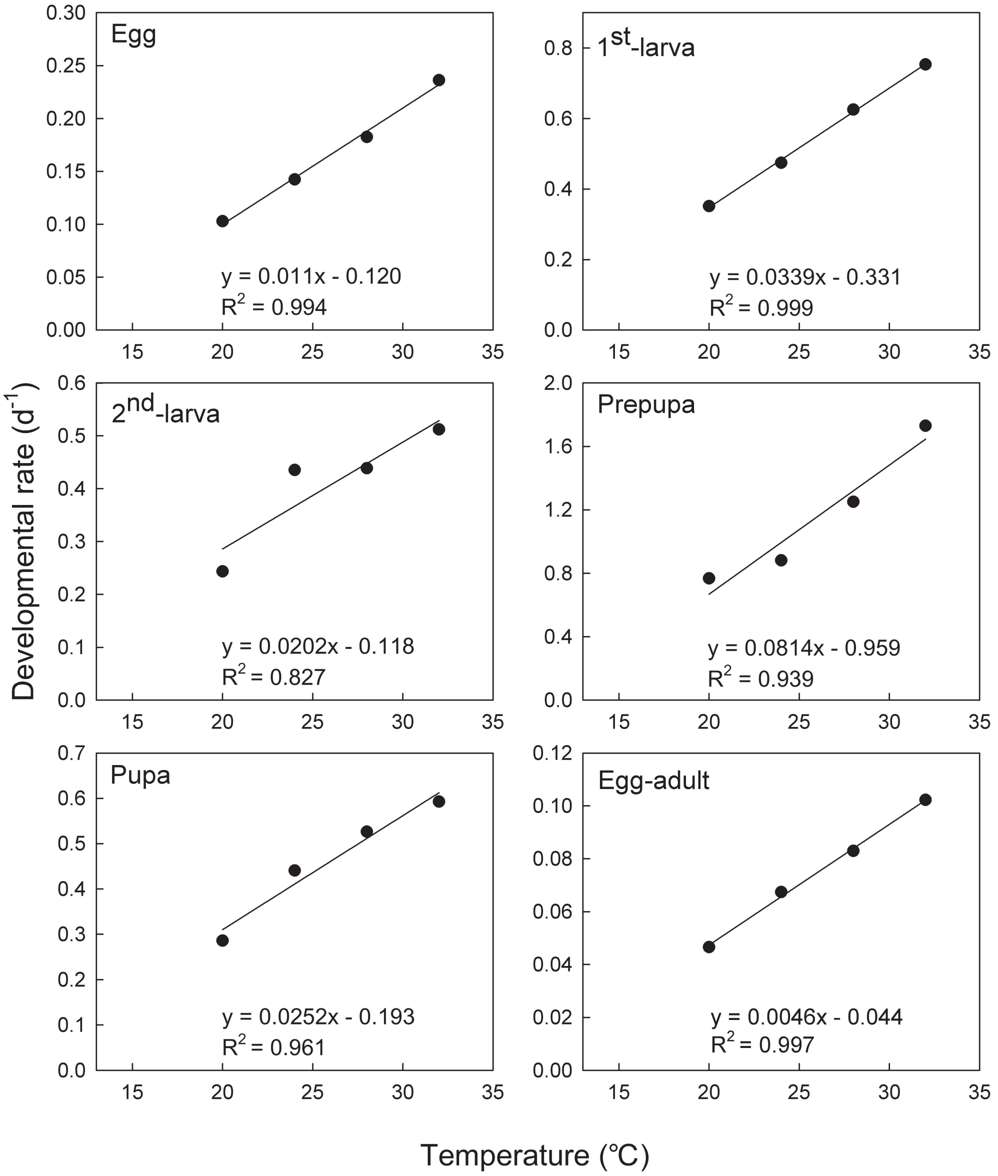

圖 1. 溫度與檬果上小黃薊馬發育速率之直線關係。

Fig. 1. Linear regressions between temperature and developmental rate of *Scirtothrips dorsalis* at 20–32℃ on mango.

2010b)，在小黃薊馬發生高峰期，選用較長效及速效殺蟲劑，將幼蟲或成蟲徹底防治，減少成蟲產卵於葉片，可以避免薊馬在田間族群大量增長，及減少對檬果之危害。

在 4 個恆溫下觀察薊馬產出之卵發育為成蟲之雌雄性比 (♀ : ♂) 均大於 1，意即產出雌性子代較多，在恆溫 24℃及 28℃下的子代雌雄性比更達 2.5 以上。由於雌蟲繁衍後代延續族群，雌蟲比例增加將加速田間族群的增長，估測在 20–32℃，溫度適合小黃薊馬在檬果繁殖，尤其是 24–28℃時將產下更多的雌蟲，田間發生數量增加，危害檬果更為嚴重。

雌、雄成蟲平均壽命在 20℃時為 26.3 d 及 17.3 d，32℃時則縮短為 13.2 d 及 8.4 d，

在 28℃時雌雄兩者平均壽命較略長於飼育在 24℃者 (表 3)，相關研究文獻亦指出小黃薊馬雌成蟲壽命隨溫度增加而縮短 (Tatara 1994; Shibao 1996)。Tatara (1994) 研究在 25℃時，以珊瑚樹、茶及溫州蜜柑飼育小黃薊馬的平均成蟲壽命分別為 23.7、27.5 及 4.0 d，除飼育於蜜柑者，其平均壽命均長於本研究 4 個恆溫飼養之薊馬成蟲。在 26℃時，以玫瑰或番椒飼育小黃薊馬之雌雄壽命分別為 19.0、13.8 d 及 18.8、15.8 d (Seal *et al*. 2010)，其雌蟲平均壽命與本試驗以檬果飼育於 24℃及 28℃之結果 (表 3) 相近。顯示，溫度與寄主植物確實會影響成蟲壽命長短。

觀察小黃薊馬繁殖力結果，在 28℃時，平均每一雌蟲每日產卵量及一生總產卵量均較飼育在其他恆溫之薊馬高 (表 3)。在其他寄主上的產卵量相關研究結果，以珊瑚樹飼育在 14.5、19.0、25.0 及 29.5℃恆溫下，平均總產卵量依序分別為 46、49、41.5 及 33 粒；25℃時飼以茶葉者，一生平均產下共 27 粒卵，飼以溫州蜜柑者平均產 1.4 粒卵 (Tatara 1994)。綜合往昔研究結果，在各恆溫下飼以不同植物，其平均產卵量均低於本試驗 28℃時之小黃薊馬產下 74 粒卵，顯示以檬果葉為食物較飼以珊瑚樹、茶及溫州蜜柑之薊馬繁殖力高。

以直線迴歸模式分析溫度與檬果上小黃薊馬發育之關係，顯示在溫度 20–32℃時，溫度與發育速率呈正相關，藉由該方程式估算其由卵發育至成蟲之臨界低溫為 9.6℃ ± 0.2℃，發育總積溫為 219.1 日度。小黃薊馬在不同寄主之發育臨界低溫及發育總積溫之相關研究結果，在珊瑚樹為 9.7℃及 265 日度；葡萄為 8.5℃及 294.1 日度 (Tatara 1994; Shibao 1996)。小黃薊馬在不同寄主上發育臨界低溫相近，但因所需發育總積溫較低，顯示小黃薊馬若在同一溫度條件下，在檬果上發世代數高於葡萄及珊瑚樹上者。由於本研究之試驗溫度在 20–32℃，所估得的臨界低溫 9.6℃落於實驗溫度線性範圍外，顯示結果可能有偏低或偏高 (Lai & Lin 2005)。未來試驗將增加低溫的觀察試驗組數，並進行非線性模式的分析，估算其發育臨界低溫、發育最適溫度及發育臨界高溫，可以更合理解釋溫度與小黃薊馬發育的關係。

台灣檬果主要產區在台南、高雄及屏東，小黃薊馬在檬果抽新梢、花穗及小果期發生嚴重，由 2010 年在台南玉井愛文檬果小黃薊馬監測結果顯示，其族群在 1 月時密度驟增後維持在高密度波動，至 6 月下降後則處於低密度之狀態 (Lin *et al*. 2010a)。根據中央氣象局網站從 2008–2012 年氣候統計資料 (http://www.cwb.gov.tw/V7/)，台南地區每月平均氣溫最低時大多發生在 1–2 月，為 15–18℃，均高於小黃薊馬的發育臨界低溫 (9.6℃)，顯示小黃薊馬在台南地區檬果上全年均可發育繁殖。不考慮食物來源、降雨、天敵等對族群密度影響，以中央氣象局每月均溫統計台南地區每年總積溫在 8,750–9,000 日度，估算小黃薊馬全年約可發生 40 個世代，但實際上在田間小黃薊馬族群發生必定受到生物與非生物之環境因子所影響，而在族群數量或發生世代數受到一定的抑制。Tatara (1994) 研究估算小黃薊馬在日本年可發生 8 代；以地理氣候軟體工具 (NAPPFAST) 估測新入侵美國佛羅里達州小黃薊馬族群年發生達 18 代 (Nietschke *et al*. 2008)，顯示台灣的氣候條件較日本及美國更適合小黃薊馬發育與繁殖，亦印證近年來小黃薊馬對台灣作物危害嚴重之情況。

若針對小黃薊馬喜好植物幼嫩組織之生活習性，台南地區檬果抽新稍開花及小果期發生在 12 月至翌年 4 月，每年在這期間之根據中央氣象局之資料統計台南地區有效積溫約為 3,100 日度，估算在 5 個月期間該地區之小黃薊馬約可發生 14 個世代；每年 1–4 月之每個月依氣象資料統計有效積溫約 600–750 日度，估算台南地區每月小黃薊馬發生約 3–4 代；5–6 月為檬果套袋成熟期，較不易受薊馬危害。故在 2–6 月玉井地區月平均溫度在 20–28℃，適合小黃薊馬之發育與繁殖，因而促使小黃薊馬田間密度之上升。由本試驗觀察結果分析，20、24 及 28℃時小黃薊馬孵化率、存活率均達 80% 以上，且平均可產下 40 粒卵以上，對照玉井檬果園小黃薊馬族群變動情形 (Lin *et al*. 2010a, 2010b)，其蟲口密度

自 1 月下旬開始由每一黏板數隻上升至數百隻，進入發生高峰期維持至 5 月下旬，實驗室觀察結果分析與實際田間發生情形相符，顯示溫度在 20–28℃，確實為本薊馬適合生存發育與繁殖範圍。根據中央氣象局資料顯示，台南地區 7–9 月月平均溫度 27–29℃，月總積溫為 820–920 日度，估計每月仍可發生 4 代。但實際田間則呈低族群密度，其原因為此時已進入檬果採收期，植株上大多為老葉不適合薊馬棲息取食。此外，本研究在 32℃時，小黃薊馬未成熟期累積死亡率較 28℃時增加，繁殖力亦下降，而台南地區 7–9 月每日最高氣溫超過 30℃多達 25 d 以上，因此食物不夠充足及溫度已接近不適其發育繁殖，為小黃薊馬在檬果上夏季族群維持低密度原因之一；而在檬果採收後，植株會進行剪枝促使新梢發生，故在 9 月後小黃薊馬在檬果上會有一發生高峰期。

本研究探討溫度對檬果小黃薊馬之存活率、發育速率及繁殖量的影響，利用直線回歸模式僅能估算其發育臨界低溫與發育總積溫，藉以分析發生世代數。然溫度與生物的發育速率並非永遠呈直線關係，生物對於低溫和高溫會反應不同生理現象，低溫會限制生物的生活及發育，但相對增高至某一範圍時也會限制其生長發育，必須以非線性回歸模式方能估算其發育臨界高溫及致死溫度 (Lai & Lin 2005)。此外，回歸模式僅能分析溫度對發育速率的影響，並無法說明溫度對於族群增長的影響。必須加入單位時間內之存活、死亡率及繁殖量等數據，以分析其生命表 (life table)，求得各族群介量，可模擬分析在田間族群增長模式。Seal *et al*. (2010) 報導小黃薊馬在番椒及玫瑰上的族群介量 (population parmeters)，在 26℃時其內在自然增殖率 (intrinsic rate of increase) 依序為 0.09 (d^{-1}) 及 0.08 (d^{-1})，由此估算小黃薊馬族群數量倍增時間 (double time) 約 8–9 d。本文作者等將進一步分析小黃薊馬發育臨界高溫、致死溫度及生命表，以提供更多更精確的資訊供檬果小黃薊馬防治上的參考應用。

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Effects of Temperature on Development and Reproduction of *Scirtothrips dorsalis* Hood on Mango

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Abstract

Chen, Y. J., F. C. Lin, Y. C. Chiu, and H. T. Shih. 2013. Effects of temperature on development and reproduction of *Scirtothrips dorsalis* Hood on mango. J. Taiwan Agric. Res. 62(4):351–359.

The chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), is one of key pests of mangoes (*Mangifera indica* Linn). Its life history was measured at constant temperatures of 20, 24, 28, and 32℃ on mango for evaluating the effect of the temperatures on development and fecundity of chilli thrips. Results showed that the survival rate of immature stages exceed 80% at four temperatures. The total developmental time decreased as the temperatures increased from 20, 24, 28, to 32℃ were 21.5, 14.8, 12.1, and 9.8 days, respectively; the mean longevity of the female was 26.3, 17.4, 19.8, and 13.2 days; and the mean fecundity per female was 37.3, 38.4, 74.0, and 40.0 eggs. The linear models adequately described the relationship between temperature and developmental rates for immature stages of chilli thrips. Thus, thrips required 219.1 degree-days to complete development from egg to adult stage, above a low threshold of 9.6℃ as estimated by linear regression. According to annual effective accumulated temperatures in Tainan climate condition, chilli thrips are expected to have about 40 generations per year, and the outbreak of population density appear from February to June in Tainan mango orchard.

Key words: *Scirtothrips dorsalis* Hood, Chilli thrips, Mango, Development, Reproduction.

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番椒斑駁病毒之鑑定與發生調查

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摘要

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甜椒 (*Capsicum annuum*) 葉片出現嵌紋及扭曲變形疑似病毒感染的病徵，經直接法 ELISA 檢測，這些樣本可與辣椒葉脈斑駁病毒 (*Chilli veinal mottle viru*; ChiVMV) 的抗體反應，且汁液接種可感染 *Nicotiana benthamiana*，但 *N. benthamiana* 中的病毒卻不與 ChiVMV 的抗體反應，也不感染紅藜、奎藜。經汁液系列稀釋後接種，可分離得一病毒純系，將其接種保存於 *N. benthamiana* 上，經寄主範圍試驗，結果只感染茄科植物，回接甜椒即在葉片出現嵌紋及扭曲變形病徵。抽取接種的 *N. benthamiana* 葉片之總量 RNA，利用 potyvirus 的簡併式引子 (HRP5/dT) 進行反轉錄聚合酵素連鎖反應，可增幅得到 1.3 kb 的 DNA 片段。進行選殖及定序分析並與 GenBank 之核苷酸序列比對，與番椒斑駁病毒 (*Pepper mottle virus*; PepMoV) VB 分離株的鞘蛋白基因 (Access No. AB126033) 相同度達 100%，因此鑑定本病毒是一株 PepMoV。純化上述接種繁殖於 *N. benthamiana* 的病毒分離株 (PepMoV-TW1) 製備多元抗血清，利用其抗體以 ELISA 進行病毒偵測，自 2007 至 2011 年採自台灣各地 969 個甜椒樣本中有 20% 可測到 PepMoV，且多數與 ChiVMV 或番椒葉脈斑駁病毒 (*Pepper veinal mottle virus*; PVMV) 複合感染。因 PepMoV 與馬鈴薯病毒 Y (*Potato virus Y*; PVY)、PVMV 及 ChiVMV 等病毒有血清關係且彼此產生交互反應，使以往 ELISA 的結果之判別及病毒種類鑑定受到干擾，且甜椒中病毒複合感染的情況極為普遍，因此推測 PepMoV 可能之前就已存在於台灣。

關鍵詞：番椒斑駁病毒、鞘蛋白、抗血清。

前言

番椒 (*Capsicum annuum* L.) 屬茄科番椒屬，原產於南美洲秘魯和中美洲墨西哥一帶。哥倫布發新大陸後，將番椒傳入西班牙和歐洲，經歐洲傳入印度，17 世紀始傳到中國。台灣甜椒栽培始於 1960 年後，由美國引進栽培。危害甜椒的病害種類眾多，主要有疫病、青枯病、細菌性斑點病、炭疽病、白絹病、白粉病、果實腐敗病、根瘤線蟲和病毒病害等 (Green & Kim 1991)。番椒作物病毒病害田間複合感染的情形相當普遍，台灣已發生的病毒包括：煙草嵌紋病毒 (*Tobacco mosaic virus*; TMV)、番茄嵌紋病毒 (*Tomato mosaic virus*; ToMV)、番椒微斑駁病毒 (*Pepper mild mottle virus*; PMMoV) (Green *et al*. 1987; Green & Wu 1991)、胡瓜嵌紋病毒 (*Cucumber mosaic virus*; CMV) (Hsu *et al*. 1989)、馬鈴薯病毒 Y (*Potato virus Y*; PVY) (Lee 1970)、番椒葉脈斑駁病毒 (*Pepper veinal mottle virus*; PVMV) (Cheng *et al*. 2009)，辣椒葉脈斑駁病毒 (*Chilli veinal mottle virus*; ChiVMV) (Tsai

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et al. 2005)、番椒斑駁病毒 (*Pepper mottle virus;* PepMoV) (Cheng *et al*. 2011)、番茄斑點萎凋病毒 (*Tomato spottd wilt virus*; TSWV) (Zheng *et al*. 2010)、番椒黃化病毒 (*Capsicum chlorosis virus*; CaCV) (Huang *et al*. 2010) 和番茄黃化捲葉病毒 (*Tomato yellow leaf curl virus*; TYLCV) (Shih *et al*. 2010)。除了 TMV、ToMV 和 PMMoV 由機械傳播外，其餘病毒均可經由機械操作和媒介昆蟲傳播，其中 CMV、PVY、PVMV、ChiVMV 及 PepMoV 可經由蚜蟲傳播，TSWV 和 CaCV 經由薊馬傳播，番茄黃化捲葉病毒只經由銀葉粉蝨 (*Besisia argentifolii*) 傳播。

PepMoV 是 Potyviridae 科 Potyvirus 屬的一員 (Shukla *et al*. 1994; Ward *et al*. 1995)，病毒顆粒呈略彎曲長桿狀，長度約為 680–900 nm，基因體約為 10 kb。主要危害茄科作物。媒介蚜蟲以非永續性傳播 (Shukla *et al*. 1994)，田間也可經由摘心採果等農事操作在植株間傳佈。PepMoV 首先報告於美國佛羅里達 (Zitter 1972; Purcifull *et al*. 1975)、亞利桑那 (Nelson & Wheeler 1972, 1978)、加州 (Nelson & Wheeler 1978; Vance *et al*. 1992)、德州 (Villalon 1975)、喬治亞州 (Benner *et al*. 1985) 與新墨西哥州 (Rodríguez-Alvarado *et al*. 2002) 等地，之後在夏威夷 (Melzer *et al*. 2012) 與古巴 (Quiñones *et al*. 2011) 亦有發現此病毒的報告。PepMoV 在亞洲首見於日本 (Ogawa *et al*. 2003) 與韓國 (Lee *et al*. 2004)，台灣也發現此病毒 (Cheng *et al*. 2011)。本研究將針對 PepMoV 台灣分離株之寄主範圍、抗血清製備與鞘蛋白序列分析並初步調查本病毒在番椒上之發生情形。

材料與方法

病毒之分離與寄主範圍測定

2006 年 8 月自南投縣埔里鎮與 2008 年自台南縣後壁鄉採集葉片呈現嵌紋、扭曲變形疑似病毒感染之甜椒樣本，這些樣本利用酵素連結免疫分析 (Enzyme-linked immunosorbent assay; ELISA) 檢測時都與辣椒葉脈斑駁病毒 (*Chilli veinal mottle virus*; ChiVMV) 的抗血清正反應。採集自南投縣埔里鎮彩色甜椒葉片呈現嵌紋及植株矮化之疑似病毒感染病徵，取其葉片以 50 倍量 (W/V) 磷酸緩衝液 (0.01M $KHPO_4$, pH 7.0) 研磨後，接種於灑有 400 目金剛砂之圓葉煙草 (*Nicotiana benthamiana*) 葉片。出現嵌紋病徵之 *N. benthamiana* 葉片以 10 倍量磷酸緩衝液研磨後，以磷酸緩衝液系列稀釋後接種於 *N. benthamiana* 葉片，觀察接種成功比例。

分離病毒時，以 500 倍量磷酸緩衝液研磨後，接種於 *N. benthamiana* 葉片，待其葉片出現病徵後，採取其中一株的葉片重複上述稀釋及接種步驟，如此重複 3 次，選擇最後出現嵌紋病徵之一株煙草視為一分離株。單一分離株接種於 *N. benthamiana* 及甜椒 (*C. annuum*)，作為本試驗接種源或於浸泡於 50% 甘油中置於 -20℃長期保存。

取罹病 *N. benthamiana* 葉片粗汁液作為接種源，依前述方法接種藜科 (Chenopodiaceae)、龍膽科 (Gentianaceae) 和茄科 (Solanaceae) 等 4 科 15 種植物 (表 1)，置於溫室中連續 6 wk 觀察病徵發展，並摘取葉片進行 ELISA 檢測。

病毒之純化與抗血清製備

以接種經過約 15 d，出現病徵的 *N. benthamiana* 葉片作為病毒純化材料，依 Gonsalves & Ishii (1980) 的方法進行純化但略加修改。每克病葉加入 2 mL 0.5 M PE 萃取緩衝液 (pH 7.5) 以果汁機打碎，過濾後加入氯仿及四氯化碳 (各 0.5 mL g^{-1}) 淨化，經低速離心去除綠色殘渣。上層液加入 8% PEG6000，於 4℃下攪拌後，經低速離心取其沉澱物，以 0.1 M PE 緩衝液 (pH 7.0) 懸浮，再經低速離心去除綠色殘渣。上澄液加入 8% PEG 及 0.3 M NaCl，攪拌後經低速離心，沉澱物以 0.1 M PE 緩衝液懸浮，同樣經過低速離心去除殘渣後加入 15% (W/V) 硫酸銫 (Cs_2SO_4)，溶解後取此溶液加至下層墊有 53% 硫酸銫之 PE 緩衝液中，離心 16 h。吸取綿密狀病毒層，以 0.1 M PE 緩衝液透析後以光譜儀 (Hitachi UV-2000 spectrophometer，日本) 於波長 320 nm

至 200 nm 自動掃描，並依 260 nm 波長的吸收光度計算病毒濃度。純化所得之病毒作爲委外製備抗血清之用。

以純化病毒爲抗原，委外製備抗血清，再以所得抗血清純化免疫球蛋白 (IgG)，作爲 ELISA 或 Western blotting (Clark & Adem 1977) 之用。

酵素連結免疫分析 (ELISA)

本試驗参照以往之研究報告進行 (Clark & Adem 1977)，取 0.1 g 之罹病葉組織，以 3 mL 之 15 mM 碳酸鈉緩衝液 (sodium carbonate buffer, pH 9.6) 研磨均勻後，加入 ELISA 反應盤內，每樣品二個重複，置於 37℃生長箱 4 h 進行覆膜反應 (coating reaction)；然後以 PBST 緩衝液 (137 mM NaC1, 1.5 mM KH_2PO_4, 1 mM Na_2HPO_4, 0.05% Tween 20, pH 7.4) 沖洗 3 次；其次加入供試抗體後，放置在 37℃定溫箱反應 2 h；再以 PBST 緩衝液沖洗 3 次後，加入已溶於磷酸緩衝液 (137 mM NaC1, 1.5 mM KH_2PO_4, 1 mM Na_2HPO_4, 0.05% Tween 20, pH 7.4) 之山羊抗兔二次抗體 (Goat anti-Rabbit immunoglobin, Jackson, West Grove, PA, 100 μL 孔$^{-1}$)，置於 37℃之定溫箱中反應 2 h；最後再以 PBST 緩衝液沖洗 4 次後，再以 μL 孔$^{-1}$ 之比例加入濃度爲 1 mg mL^{-1} 之鹼性磷酸酶酵素基質 (ρ-NPP, Amresco, Solon Ind., Ohio, USA) 進行呈色反應。反應後 20 至 30 min，以 ELISA 讀值儀 (PTI max micro plate reader, Mlolecular Devices, Sunnyvale, CA) 讀取波長 405 nm 下之吸收值，作爲評估病毒濃度高低之依據。樣品讀値大於健康葉片之 2 倍者，視爲正反應。

西方漬染法 (Western blotting)

取 0.1 g 供試之病葉組織，以液態氮磨碎後，加入 200 μL 之樣品處理液 (75 mM Tris-HCl, 10 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 30% glycerol, 6% SDS, 9% β-Mercaptoethanol, 0.015% Bromophenol blue)，研磨液加熱處理後經 SDS-PAGE 電泳，膠體上之蛋白轉漬於 PVDF 膜 (Millipore Co., MA, USA)，再以病毒抗血清進行漬染反應。

RNA 之抽取及反轉錄—聚合酶連鎖反應

取 100 mg 的病葉，利用總量 RNA 純化試劑組 (Total RNA extraction kit, Qiagen, Hilden, Germany) 進行 RNA 之萃取，得到總量 RNA。病毒 RNA (viral RNA) 之抽取則取純化病毒以病毒 RNA 純化試劑組 (Viral RNA extraction kit, Qiagen, Hilden, Germany) 進行萃取。以純化所得之 RNA 進行反轉錄聚合酶連鎖反應 (reverse transcription-ploymerase chain reaction; RT-PCR)，RT-PCR 以單一步驟 RT-PCR 試劑組 (Genemark Co., Taichung, Taiwan) 進行，25 μL 的反應液中加入 1 μL 的 RNA，各 2.5 μL 之 20 μM 簡併式引子 Hrp5/Pot1 (Pappu *et al*. 1993)。RT-PCR 之進行於於熱循環反應儀 (GeneAmp model 2400, Perkin-Elmer Co., Norwalk, CT) 中，設定反應程序爲 50℃下進行反轉錄 30 min，94℃變性 1 min 30 s；後進行 30 個 PCR 循環反應：94℃下變性 1 min，50℃下鍊合 1 min 30 s，72℃下聚合 2 min，最後一個循環之 72℃聚合反應延長爲 6 min。反應產物以 1.2% 電泳瓊膠 (SeaKem, Agarose, Cambrex Bio Science Rockland, Inc., Rockland, ME USA) 進行分析。

基因選殖及定序分析

RT-PCR 增幅所得之 DNA 片段以小量膠體 DNA 回收組 (Genemark Co., Taichung, Taiwan) 回收 DNA，以 TOPO TA-PCR Ⅱ Cloning Kit (Invitrogen Co., Carlsbad, CA, USA) 進行選殖。篩選得到之選殖株抽取質體經限制酶 EcoRI 酶切確認插入 DNA 大小後，用於進行定序分析。定序反應委託明欣生物科技公司 (City, Country) 進行。定序所得之核苷酸序列以 Vector NTI Suite (InforMax, Inc. Wesconsin, USA) 分析軟體進行連接，並與 GenBank (www.ncbi.nlm.nih.gov) 上已登錄之核苷酸序列進行分析比對，再以 PALM: Phylogenetic Reconstruction by automatic Likelihood Model Selector (http://palm.iis.sinica.edu.tw) (Chen *et al*. 2009) 作成親緣關係樹狀圖。

病毒發生調查

利用本實驗製備的抗血清所抽取的純化免疫球蛋白 (IgG)，加上實驗室先前製備的感染番椒病毒 TMV (亦與 ToMV、PMMoV 反應)、CMV、PVY、ChiVMV (亦與 PVMV 反應)、TSWV、CaCV 等病毒之 IgG，針對各縣市採集之番椒疑似病毒病害標本進行 ELISA，調查病毒在番椒上的發生情形。

結果

病毒分離與寄主植物測定

利用罹病葉片汁液進行接種試驗，結果在紅藜 (*Chenopodium amaranticolor*) 及奎藜 (*Chenopodium quinoa*) 接種葉出現黃色圓斑，在 *N. benthamiana* 上位葉出現嵌紋病徵。再次以 ChiVMV 抗血清檢測，造成紅藜及奎藜葉片圓斑的病毒仍呈現正反應，但造成 *N. benthamiana* 嵌紋病徵的病毒則呈現負反應，因此發現感染 *N. benthamiana* 的病毒與感染紅藜及奎藜者不同，不為 ChiVMV。

利用 *N. benthamiana* 病葉汁液再接種，於紅藜、奎藜接種葉皆無黃斑出現，甜椒則出現葉片嵌紋及扭曲變形病徵。因感染 *N. benthamiana* 之病毒不感染紅藜或奎藜，無法以單斑分離純系病毒，因此以系列稀釋法分離純化病毒。取 *N. benthamiana* 病葉以 10 倍量磷酸緩衝液研磨，再以 10 倍、100 倍與 1,000 倍緩衝液稀釋接種於 *N. benthamiana*，其中 100 倍稀釋接種的 20 棵植物都出現嵌紋病徵，1,000 倍稀釋接種的 20 棵中只有 1 棵出現病徵，因此連續 3 次以 500 倍稀釋接種源進行病毒分離，得到埔里分離株 (PepMoV-TW1) 與後壁分離株 (PepMoV-HB1)，接種於 *N. benthamiana* 上繁殖，作為本試驗接種源或取病葉組織浸泡於 50% 甘油中置 -20℃長期保存。

機械接種 3 科 15 種植物中，PepMoV-TW1 只感染茄科寄主，不感染藜科、葫蘆科及豆科的供試植物 (表1) (圖 1)，PepMoV-TW1 除了感染甜椒、辣椒及各品種煙草外，也感染矮牽牛 (Petunia × hybrid)、酸漿 (*Physalis angulate*) 和番茄 (*Solanum lycopersicum*)，在這 3 種植物上雖只造成植株矮化，但以 ELISA 檢測確認病毒感染。PepMoV-TW1 除了在甜椒、辣椒和 *N. benthamiana* 葉片造成明顯嵌紋和植株矮化之外，其他測試植物只造成矮化，葉片未觀察到明顯病徵。PepMoV-HB1 之寄主範圍與 PepMoV-TW1 相同，造成病徵亦相似。

與病毒之純化與抗體之製備

接種 PepMoV-TW1 的 *N. benthamiana* 葉片經上述純化步驟後所得病毒，其 A260/A280 比值為 1.19，估算病毒回收量每 100 g 煙草之收量約 3.2 mg 純化病毒。製備所得抗血清其純化之 IgG，稀釋至 1 mg mL^{-1}，可再稀釋至 1,000 倍作為 ELISA 與西方漬染法之試劑。

病毒鞘蛋白分子量與西方轉漬法分析

純化的病毒、罹病甜椒與番茄經 SDS-PAGE 與西方轉漬法分析，其鞘蛋白分子量介於蛋白標誌 27 至 41 kDa 之間，推估其分子量約為 32 kDa (圖 2)，符合馬鈴薯 Y 病毒群分子量 (Shukla *et al*. 1994)。

番椒斑駁病毒與其他感染番椒之馬鈴薯 Y 病毒屬病毒之血清相關性

利用 indirect ELISA 比較台灣已知感染甜椒之 PVMV、ChiVMV 及 PVY 與 PepMoV-TW1 之血清相關性。各以 4 種病毒感染的 10 棵甜椒為樣本，分別與前述 IgG 及實驗室製備的 3 種病毒多元抗體純化所得 IgG 進行試驗，PepMoV-TW1 之 IgG 除了與病毒本身感染甜椒呈色 40 min 之後反應值已達 3.02，但與 PVY 感染甜椒反應值 1.80，與 PVMV、ChiVMV 感染甜椒反應值 0.71 與 0.69。PepMoV-TW1 與 PVY 血清相關性較 PVMV 與 ChiVMV 強 (表 2)，與 Moury *et al*. (2005) 報告相符。

PepMoV 二分離株鞘蛋白基因核苷酸之序列分析比對

利用偵測 Potyvirus 之簡併式引子 Hrp5 和 Pot1 (Pappu *et al*. 1993)，以接種 PepMoV-TW1 與 PepMoV-HB1 罹病甜椒的總量 RNA 進行 RT-PCR，反應結果以 1.2% agarose 電泳

表 1.　番椒斑駁病毒台灣分離株 TW1 與 HB1 在供試植物上之反應及與報告中其他國家分離株寄主範圍比較。

Table 1.　Symptoms on different species of plants inoculated with Pepper mottle virus from Taiwan isolates (TW1 and HB1) and other isolates reported in Japan, Korea, and USA.

	Isolate of *Pepper mottle virus*				
Plant species	TW1[z]	HB1[z]	Japan[y]	Korea[y] (Tom-sd2)	America[y]
Chenopodiaceae					
Chenopodium amaranticola	-	-	-	**	NL
Chenopodium quinoa	-	-	-	**	-
Cucurbitaceae					
Cucurbita moschata	-	-	-	**	**
Cucumis sativus	-	-	-	**	**
Leguminosae					
Phaseolus vulgaris	-	-	-	**	**
Vicia faba	-	-	-	**	**
Solanaceae					
Capsicum annuum					
Chili pepper	M[x]	M	F, D	M	D, M
Green pepper	D, M	D, M	D, M	Mo/N	D, M
Nicotiana benthamiana	M	M	-	M	**
Nicotiana rustica	D	D	-	**	**
Nicotiana tabacum	Mo	Mo	Mo	M	Mo
Petunia × hybrida	D	D	VC, Mo	**	**
Physalis angulata	D	D	-	**	Mo
Solanum lycopersicum	D	D	-	VN	Mo, D
Solanum melongena	-	-	-	**	**

[z] Isolates of Pepper mottle virus from Taiwan: TW1 and HB1.
[y] Reported Isolate from Japan (Ogawa *et al.* 2003), Korea (Kim *et al.* 2008) and America (Purcifull *et al.* 1975; Nelson & Wheeln 1978).
[x] Abbreviation of symptoms: chlorotic spot (CS), crinkle (CR), dwarf (D), necrotic lesion (NL), mosaic (M), leaf mottle (Mo), fallen leaves (F), vein clearing (VC), vein necrosis (VN), no symptom (-), and no data (**).

分析，觀察到增幅出約 1.3 kb 之 DNA 條帶。

增幅之 DNA 片段選殖至 TOPO TA 選殖套組，所得之選殖株經定序後，共有 1,350 個核苷酸，包含病毒之部分 Nib 基因、鞘蛋白基因和 3’ 非轉譯區。其中 PepMoV-TW1 部份在 GenBank 登記為 Acc. No. HQ329082，PepMoV-HB1 部 份 為 Acc. No. JX962777。PepMoV-TW1 與 PepMoV-HB1 分離株之鞘蛋白基因與 GenBabk 登錄之序列 PepMoV 鞘蛋白基因比對 (表 3)，利用 PALM 繪成親緣關係樹狀圖如圖 3。PepMov-TW1 鞘蛋白基因的 822 核苷酸與韓國的分離株 VB (Access No. AB126033) 之核苷酸相同度最高，達 100 %。

甜椒病毒病害發生調查

採集自南投、雲林、嘉義、台南、屏東、花蓮及台東的甜椒共計有 969 個樣本，檢測結果如表 4。檢測病毒中以 PVMV 感染比例最高，達 34.2%，其次為其他 3 個 Potyvirus 屬的 PVY、ChiVMV 和 PepMoV 感染比例都在 20% 以上，CMV 感染比例為 7.3%，PMMV 感染率合計達 11.8%，粉蝨傳播的 TYLCV 感染比例為 11.1%，薊馬傳播的 CaCV 與 TSWV 感染比例為 5.6% 與 9.0%，未檢測到上述病毒者 185 個樣本，佔 19.1%。檢測樣本中，PepMoV 多與 PVMV、ChiVMV 複合感染。檢測的辣椒樣本中，多為 CMV 和 PVMV、

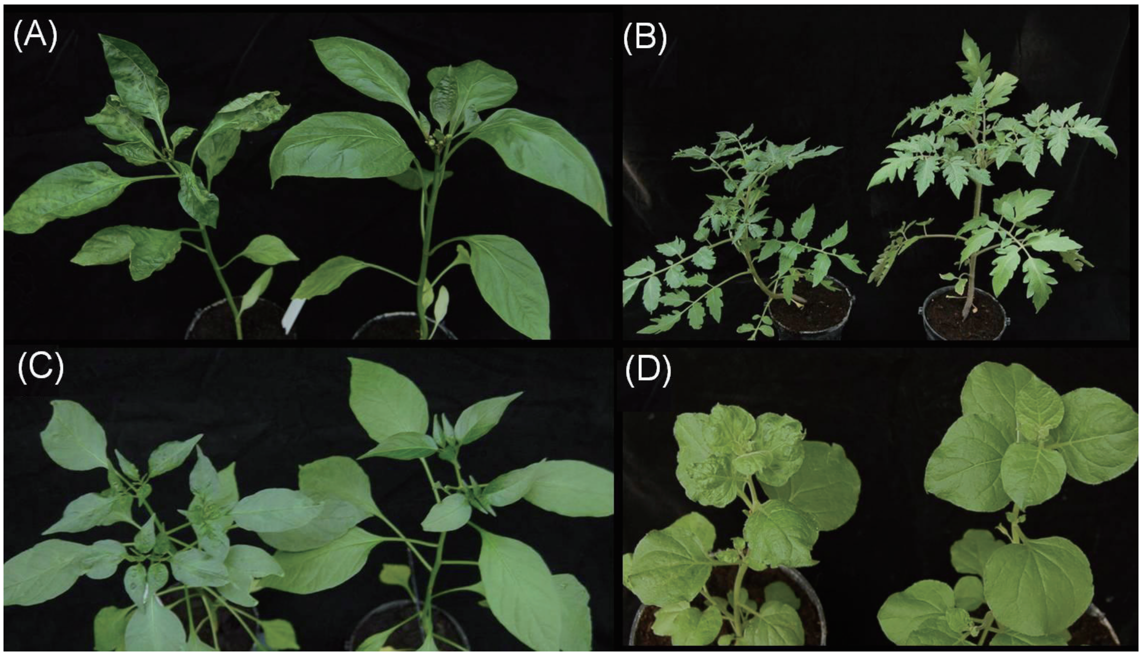

圖 1. 番椒斑駁病毒 TW1 分離株接種於甜椒 '女王星'[(A), 圖左]、辣椒 '萬里香' [(B), 圖左]、番茄 '農友 301' [(C), 圖左]、和煙草 [(D), 圖左] 所出現的病徵。(A)–(D)： 圖右為未接種對照。TW1 分離株在甜椒 (A) 上引起嵌紋及葉片扭曲病徵；在辣椒 (B) 上引起之嵌紋病徵；在番茄 (C) 上引起之矮化病徵 ; 與在煙草 (D) 上引起之嵌紋病徵。

Fig. 1. Symptoms of *Pepper mottle* virus- TW1 inoculated on green pepper cv. 'Knownyou 1501' [(A), left plant]; chili pepper cv.'Knownyou 1291' [(B), left plant]; tomato cv. 'Knownyou 368' [(C), left plant]; and *Nicotiana benthamiana* [(D), left plant]. Note symptoms of systemic mosaic and deformation on the plant of green pepper [(A), left]; systemic mosaic on chili pepper [(B), left]; drawfing on tomato [(C), left]; and systemic mosaic on *Nicotiana benthamiana* [(D), left]. Uninoculated plants [(A)–(D), right] were healthy.

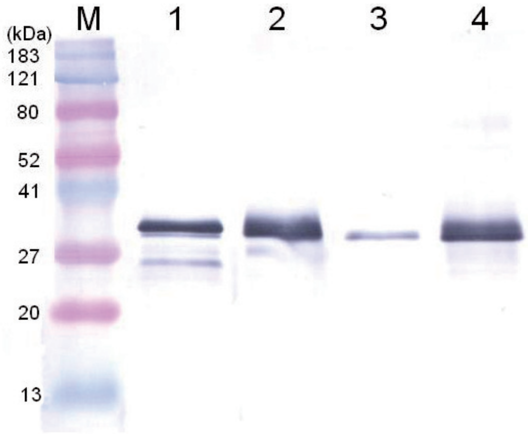

圖 2. 利用西方漬染法分析番椒斑駁病毒感染之番茄與甜椒。行 1：罹病蕃茄；行 2：罹病甜椒；行 3：未接種甜椒；行 4：純化病毒；行 M：蛋白分子量標誌。

Fig. 2. Western blotting analysis of PepMoV from inoculated plants of tomato and green pepper. Lane 1: virus inoculated on tomato; Lane 2: virus inoculated on green pepper; Lane 3: uninoculated green pepper; Lane 4: purified virus; and Lane M: protein markers.

表 2.　番椒斑駁病毒與感染台灣番椒作物其他馬鈴薯 Y 病毒屬病毒抗血清相關性。

Table 2.　Reaction of four pepper viruses with polyclonal antibodies tested by the indirect ELISA method.

	Source of polyclonal antibody			
Virus[z]	PVMV	ChiVMV	PVY	PepMoV
PVMV	+++[y]	+++	−	+,−
ChiVMV	+++	+++	−	+,−
PVY	+	+	+++	++
PepMoV	−	−	+−	+++

[z] Viruses from pepper: PVMV, *Pepper veinal mottle virus*; ChiVMV, *Chilli veinal mottle virus*; PVY, *Potato virus* Y; and PepMoV, *Pepper mottle virus*.

[y] Results of indirect ELISA: −, no serological reaction [absorbancesat 405nm (A405) < 2 times the non-inoculated control]; +, −, A405 between 2 and 5 times the non-inoculated control; +, A405 between 5 and 10 times the non-inoculated control; ++, A405 between 11 and 20 times the non-inoculated control; +++, A405 between 21 and 30 times the non-inoculated control.

表 3.　番椒斑駁病毒 PepMoV-TW1 (HQ329082) 與 PepMoV-HB1 (JX962777) 鞘蛋白基因核苷酸序列與同種病毒之比較。

Table 3.　Comparison of identities of coat protein gene nucleotide sequences of PepMoV-TW1 (HQ329082) and PepMoV-HB1 (JX962777) with *Pepper mottle virus* isolates from other countries.

	Identity (%)		
Virus isolate (Accession No.)	TW1	HB1	Country[z]
TW1 (HQ329082)	100.0	97.3	Taiwan
Vb (AB126033)	100.0	97.3	Korea
57 (EU586121)	98.1	97.9	Korea
KP-3 (AB084486)	97.8	97.6	Korea
248 (EU586125)	97.9	97.9	Korea
205187 (EU586133)	98.3	97.8	Korea
JKK (AB098560)	98.5	98.7	Japan
Ca (M96425)	97.1	96.1	America
Poamoho (JQ429788)	96.1	95.7	America
NC165 (AF227728)	83.8	83.6	America
Florida (AF501591)	94.0	94.0	America
137 (JN222367)	92.8	92.7	Cuba

[z] References for isolates from Korea (Kim *et al*. 2009), Japan (Ogawa *et al*. 2003), America (Vance *et al*. 1992; Rodríguez-Alvarado *et al*. 2002; Melzer *et al*. 2012) and Cuba (Quiñones *et al*. 2011).

ChiVMV 複合感染，比例高達 80.5%，PepMoV 亦與前述 3 種病毒之一複合感染，但測得 PepMoV 比例只有 7.5% (數據未顯示)。

討論

根據上述寄主範圍試驗、病毒的鞘蛋白分子量、血清學反應、反轉錄聚合酶連鎖反應、序列比對試驗、及親緣分析的結果，確認感染南投埔里甜椒病毒 (PepMoV-TW1) 與台南後壁甜椒病毒 (PepMoV-HB1) 是番椒斑駁病毒的分離株。利用 PepMoV-TW1 的鞘蛋白製備多元抗血清，此抗血清雖與台灣茄科作物常見的 PVY 有中等血清反應，與 PVMV 與 ChiVMV 有較輕血清反應，若同時以上述 3 種抗血清進行 ELISA 檢測，若樣本只感染 PVY、PVMV 或 ChiVMV 時，可能有讀值較低之非專一性反應。

寄主範圍測定時，PepMoV-TW1 感染植物呈現矮化或斑駁等較輕微病徵，因此接種後

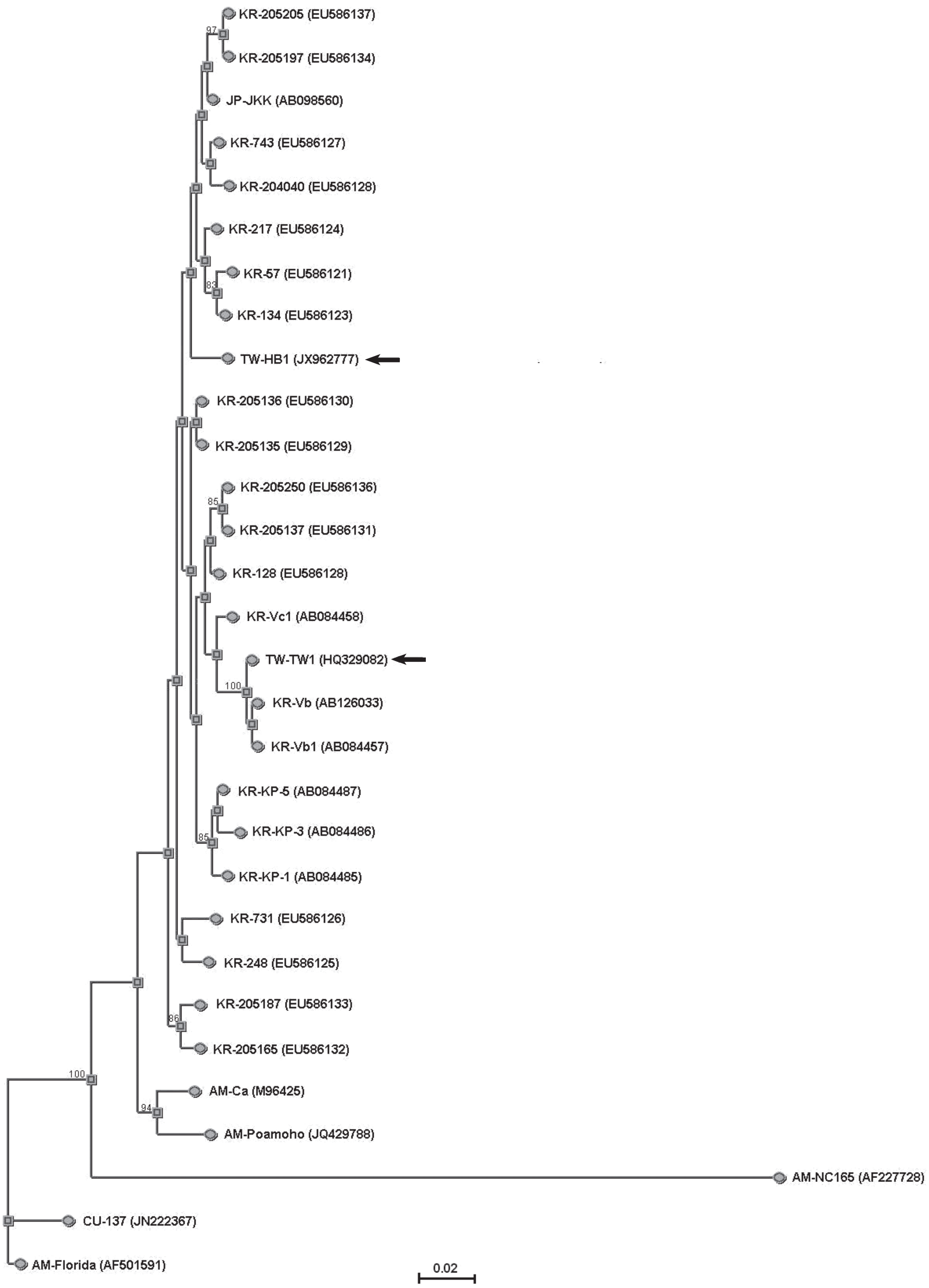

圖 3. 番椒斑白病毒鞘蛋白基因以 PALM 進行親緣關係分析之樹狀圖。

Fig. 3. Phylogenetic dendrogram of coat protein gene of PepMoV-TW1 (HQ329082) and PepMoV-HB1 (JX962777). The tree was generated by neighborhood-joining method (PALM). The numbers at the nodes indicated the levels of bootstrap support based on a neighborhood-joining bootstrap analysis of 1,000 replications.

表 4. 自 2007 至 2011 年台灣中部地區甜椒病毒病害發生概況調查。

Table 4. Occurrence of virus diseases in sweet pepper collected in central Taiwan from 2007 to 2011.

Year	No. of plants assayed	Number and percentage of plants infected with virus[z]									
		PMMV	CMV	CaCV	TSWV	PVY	PVMV	ChiVMV	PepMoV	TYLCV	ND
2007	145	15 10.3%	1 0.7%	20 13.8%	0 0%	44 30.3%	52 35.9%	40 27.6%	18 12.4%	14 9.7%	36 24.8%
2008	231	20 8.7%	20 8.7%	15 6.5%	0 0%	56 24.2%	76 32.9%	42 18.2%	51 22.1%	18 7.8%	46 19.9%
2009	282	31 11.0%	26 9.2%	13 4.6%	51 18.1%	42 14.9%	90 31.9%	50 17.7%	70 24.8%	37 13.1%	53 18.8%
2010	169	30 17.8%	19 11.2%	6 3.6%	29 17.2	50 29.6%	54 32.0%	40 23.7%	43 25.4%	11 6.5%	39 23.1%
2011	142	18 12.7%	5 3.5%	0 0%	7 4.9%	21 14.8%	60 42.3%	23 16.2%	15 10.6%	28 19.7%	11 7.7%
Total	969	114 11.8%	71 7.3%	54 5.6%	87 9.0%	213 22.0%	332 34.2%	195 20.1%	197 20.3%	108 11.1%	185 19.1%

[z] Virus diseases: PMMV, *Pepper mild mottle virus*; CMV, *Cucumber mosaic virus*; CaCV, *Capsicum chlorosis virus*; TSWV, T*omato spotted wilt virus*; PVY, *Potato virus Y*; PVMV, *Pepper veinal mottle virus*; ChiVMV, *Chilli veinal mottle virus*; PepMoV, *Pepper mottle virus*; TYLCV, *Tomato yellow leaf curl virus*; and ND, Sample did not detect the nine viruses.

再以 ELISA 確認感染。PepMoV-HB1 在接種植物上感染與否與病徵表現與 PepMoV-TW1 類似，因此表一僅以 PepMoV-TW1 呈現。PepMoV-TW1 致病性與美國的幾個分離株較相近 (Purcifull *et al*. 1975; Nelson & Wheeln 1978)，但 PepMoV-TW1 不感染 *Chenopodium amaranticola*，韓國與日本的分離株亦不感染 *C. amaranticola* (Ogawa *et al*. 2003; Lee *et al*. 2004)。測試植物中，PepMoV-TW1 感染茄子 (*Solanum. melongena*) 以外的茄科植物，雖然測試的二分離株都感染番茄 (*S. lycopersicum*) 引起矮化病徵，但田間番茄樣本未測到有 PepMoV 感染。

比對 GenBanK 登錄的鞘蛋白基因序列，台灣 2 分離株與日本 1 分離株，韓國 22 分離株的親源關係較為接近 (Kim *et al*. 2009)，與美國、古巴或夏威夷的親源關係較遠，與地理分布似呈相關，但日本與韓國分離株在寄主植物上之病原性較台灣分離株強，這些疑點需要測試更多品種、病毒分離株與病毒全長度基因體定序分析來加以釐清。PepMoV 感染的番椒樣本都為複合感染，所有供試樣本的中 PepMoV 都不感染實驗室常用的局部病斑寄主—紅藜與奎藜。因 ChiVMV 不感染 *N. benthamiana*，試驗中使用的二分離株才能順利從複合感染中分離出來，其他會感染 *N. benthamiana* 的 CMV 或 PVMV 之複合感染樣本中，PepMoV 的分離都失敗，日後將嘗試以其他產生單斑寄主如 *Datura stramonium*、*Nicotiana glutinosa* 或 *N. tabacum* 進行分離。

病害發生調查的檢測樣本中，PepMoV 多數與 PVMV、ChiVMV 複合感染。甜椒在台灣多為露天栽培，彩色甜椒則為設施栽培，不論設施栽培或露天栽培，病毒病徵植株常呈現區塊存在，甜椒為連續採果作物，且栽培過程需持續摘除側芽，推測可能去芽或採果時，刀具等造成病毒傳播。相較於甜椒，辣椒多為露天栽培。傳統上認為辣椒對病毒具有較高的抗(耐) 病性，因此栽培上較為粗放，一但栽培感病的品種，極易造成很高的病毒感染率，幾乎全園發病。檢測的樣本中，辣椒感染 PepMoV 的比例亦遠較甜椒低。

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Identification and Occurrence of *Pepper mottle virus* Isolated from Peppers in Taiwan

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Abstract

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Bell pepper (*Capsicum annuum* L.) plants exhibiting systemic leaf mosaic and malformation were collected. By DAS-ELISA, these samples were positively reacted with antibody against *Chilli veinal mottle virus* (ChiVMV). After mechanical inoculation with sap extracted from these samples, unexpected mosaic symptoms were developed on upper leaves of *Nicotiana benthamiana*. The virus infecting *N. benthamiana* was non-reacted with antibody to ChiVMV when tested by DAS-ELISA. And the virus was non-infectious to *Chenopodium amaranticolor* and *Chenopodium quinoa* tested by sap-inoculation. Pure virus isolates were obtained from infected plants of *N. benthamiana* by mechanical inoculation with serially diluted saps of infected plants. Not only pepper plants were developed the mosaic and leaf distortion symptoms, but also some species of Solanaceae plants were found to be infected by this virus in the host range test. The total RNA extracted from leaves of inoculated *N. benthamiana* was subjected to RT-PCR. The potyvirus degenerate primers (Hrp5/Pot1) were used to amplify a RT-PCR product of 1.3 kb DNA fragment, which was then cloned and sequenced. Results of nucleotide sequencing revealed that the CP gene of the isolate was 100% identical to *Pepper mottle virus* (PepMoV)-VB (Access No. AB126033) and was concluded as an isolate of PepMoV. Polyclonal antiserum against PepMoV Taiwan isolate (PepMoV -TW1) was prepared by immunizing rabbit with purified virus that has been propagated in *N. benthamiana*. Use of its antibody in ELISA for virus detection, a total of 969 sweet pepper samples were collected from different areas of Taiwan and tested in 2007–2011. About 20% of all the survey samples were identified as PepMoV infection and most of them were complexly infected with ChiVMV, or *Pepper veinal mottle virus* (PVMV). It is speculated that PepMoV might already exist in Taiwan earlier, but it was ignored because the closely serological relationships among PepMoV, *Potato virus Y* (PVY), PVMV and ChiVMV could form cross-reactions with each other and that interfered with the judgment of ELISA. Besides, the virus complex infections were very common in plants of sweet pepper.

Key words: *Pepper mottle virus*, Coat protein, Antiserum.

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成蟲飼料添加有機磷類殺蟲劑對瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] (Diptera: Tephritidae) 之毒效測試

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摘要

賴信順。2013。成蟲飼料添加有機磷類殺蟲劑對瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] (Diptera: Tephritidae) 之毒效測試。台灣農業研究 62(4):372–381。

本研究利用 8 種有機磷類殺蟲劑分別依 5 種比例添加於成蟲飼料中，評估其對瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 的毒殺效果。試驗結果顯示隨著藥劑濃度的升高，瓜實蠅的死亡率也隨著升高。僅供含毒食餌之非選擇性試驗結果顯示，含大滅松的食餌對瓜實蠅有較佳的毒殺效果，濃度 1/2000 (v/v) 時大滅松食餌在 24 h 後即可導致 85% 的瓜實蠅死亡率，在 48 h 後則達 98%，而含佈飛松的食餌對瓜實蠅的毒殺效果最差。同時供給含毒及無毒食餌的選擇性試驗結果顯示，所有供試餌劑中仍以含大滅松的食餌對瓜實蠅的毒殺效果最高，而含撲滅松或馬拉松的食餌對瓜實蠅的毒殺效果最低。殘效試驗結果顯示，賽達松食餌持效性在所有供試藥劑中最長，濃度 1/100 經 2 wk 後瓜實蠅死亡率仍可維持超過 80%。綜合而言，供試藥劑中之陶斯松、大滅松、芬殺松、賽達松與普硫松添加於餌劑對瓜實蠅有良好的毒殺效果，可作為未來瓜實蠅誘殺餌劑之添加農藥的參考。

關鍵詞：瓜實蠅、有機磷類殺蟲劑、餌劑。

前言

瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 俗稱瓜仔蜂或瓜蠅，屬於雙翅目 (Diptera)，果實蠅科 (Tephritidae)。已知其寄主植物超過 81 種以上，其中以葫蘆科 (Cucurbitaceae) 植物為主，包括苦瓜、洋香瓜、南瓜與絲瓜等，而其他作物如番茄、長豇豆和敏豆亦為寄主植物 (Dhillon *et al*. 2005)。瓜實蠅對作物主要的危害方式為幼蟲蛀食果實，但有時也會出現幼蟲啃食花器或莖部的情形 (Wang *et al*. 2000; Dhillon *et al*. 2005)。

瓜實蠅是葫蘆科作物的關鍵害蟲，成蟲產卵於植物組織中，孵化後幼蟲直接往植體內部蛀食，發育至老熟幼蟲時則跳離植株藏匿於土中化蛹，防治技術上對於這三個時期皆不易著力，因此成蟲期為主要的防治時期 (Roessler 1989; Chu 1998)。瓜實蠅成蟲的生態習性為棲息於瓜類栽培區外圍的樹林內或玉米葉片上，僅在雌成蟲產卵時才會進入瓜園 (Wen 1985)。在食物方面，除了水之外，成蟲皆需要碳水化合物作為能量來源，且需取食蛋白質以促進其性成熟 (Bateman 1972)。

目前國內報導用於瓜實蠅上的防治技術有果實套袋隔絕產卵 (Fang & Chang 1986; Fang *et al*. 1988; Fang 1989)、番石榴果實網袋包誘殺 (Ho *et al*. 2003)、黃色黏板誘殺 (Liu & Chang 1995; Lu 1997)、0.02% 賜諾殺濃餌劑誘殺配合清除園內被害果、懸掛 85% 克蠅香

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混合溶液或 95% 克蠅溶液與 50% 芬殺松乳劑以 10：1 比例混合的誘引盒誘殺雄蟲以及 25% 馬拉松可濕性粉劑加入蛋白質水解物點噴植株周圍誘殺成蟲等 (Fei *et al*. 2010)。為了減少噴灑農藥對環境與生態造成影響，將農藥添加於誘引劑內在園區周圍以點噴或懸掛餌片的方式進行防治，相較於大面積噴灑農藥，被認為是對環境較友善的果實蠅類害蟲族群管理方式 (Roessler 1989; Jiji *et al*. 2010)。國內對瓜實蠅防治研究工作多集中於誘引物質的探討與開發 (Liu & Chang 1995; Lu 2002; Wang & Liu 2010)，而將不同種類的農藥添加於誘引劑內，評估對瓜實蠅防治效果的研究篇幅相對較少 (Fang & Chang 1986; Liu *et al*. 1997; Wang & Liu 2002; Dong & Chen 2004; Lai & Yang 2012)。

有機磷類殺蟲劑 (organophosphates) 主要殺蟲機制是抑制乙醯膽鹼酯酶作用，導致昆蟲過度興奮、痙攣或麻痺等而死亡 (Savolainen 2001)，其同時具有觸殺與胃毒作用。目前國內植物保護手冊推薦防治瓜實蠅的有機磷類殺蟲劑，並不能噴施於全株植物上，而推薦可直接噴施於瓜類植株上防治其他害蟲的有機磷類殺蟲劑僅有 43% 佈飛松乳劑與 50% 馬拉松乳劑 (Fei *et al*. 2010)。有機磷類殺蟲劑為農民使用頻率較高且較易取得的藥劑種類，且普遍單位容量的價格相較於其他作用機制的殺蟲劑便宜，但是其廣效性的殺蟲效果對於生態環境的傷害與對哺乳類動物的毒害亦具有相對較高的風險。為了避免農民錯誤使用有機磷類殺蟲劑防治瓜實蠅，如將需要點噴於植株周圍的藥劑或未推薦的藥劑直接噴施在植株或瓜果上等，若能以較安全簡便的方式利用有機磷類殺蟲劑來防治瓜實蠅，除了可以減少瓜果殘留農藥過量的風險外，並且降低有機磷類殺蟲劑直接噴施對環境、天敵與人體的傷害。

本研究擬將有機磷類殺蟲劑與瓜實蠅食物誘引劑相結合製成餌片，以較簡便與較低的農藥成本，提高對瓜實蠅的防治效益，並且以較安全的施用方式降低對環境的影響。挑選市售 8 種常見的有機磷類殺蟲劑，分別以 5 種不同比例與食餌均勻混合，探討其對瓜實蠅接觸或取食後所造成的致死效果。藉由非選擇性試驗 (提供含毒食餌與水供瓜實蠅取食)、選擇性試驗 (同時提供含毒與不含毒食餌供瓜實蠅選擇) 與殘效試驗，篩選出適合添加於食餌中的藥劑與其比例，以冀未來能將有機磷類殺蟲劑以對環境較友善的運用方式，作為瓜實蠅整合性管理技術應用參考。

材料與方法

供試昆蟲來源

將受瓜實蠅 (*B. cucurbitae*) 為害的胡瓜，置於溫室內的白色飼養帳 (60 cm × 60 cm × 60 cm)，伺瓜實蠅羽化之後，以二級砂糖和酵母水解物 (MP Biomedical, Irvine, CA) (4：1) 及沾水棉花供瓜實蠅成蟲取食，並且以南瓜、苦瓜或花胡瓜供其產卵與幼蟲取食，以建立供試蟲源。

試驗藥劑配製

食餌：將二級砂糖、酵母水解物與水以 4：1：5 (w/w/w) 的比例混合製備成食餌 (Hsu *et al*. 2004)，以棉片 (3.5 cm × 3.5 cm × 0.8 cm) 吸收 12 mL 的食餌作為食餌棉片。

供試藥劑：40.8% 陶斯松乳劑 (chlorpyrifos，惠光股份有限公司)、44% 大滅松乳劑 (dimethoate，興農股份有限公司)、50% 撲滅松乳劑 (fenitrothion，興農股份有限公司)、50% 芬殺松乳劑 (fenthion，興農股份有限公司)、50% 馬拉松乳劑 (malathion，興農股份有限公司)、50% 賽達松乳劑 (phenthoate，台灣日產化工股份有限公司)、43% 佈飛松乳劑 (profenofos，先正達股份有限公司) 與 50% 普硫松乳劑 (prothiofos，惠光股份有限公司)，分別由農藥販售業者處購得。

含毒食餌配製方法與比例：8 種藥劑分別依比例 1/100、1/200、1/500、1/1000 與 1/2000 (v/v) 添加於食餌中均勻混合，24 h 後以棉片 (3.5 cm × 3.5 cm × 0.8 cm) 吸收 12 mL 的混合液作為供試含毒食餌棉片，進行試驗工作。

毒效試驗

爲納入田間環境因子如光照和溫度等對藥效的影響，本試驗乃於溫室中進行。於試驗前 72 h 由飼養帳逢機取出 60–70 隻瓜實蠅成蟲移至方型飼養籠 (30 cm × 30 cm × 30 cm) 內，以食餌棉片進行餵食，於試驗開始前清出自然死亡的成蟲。

非選擇性試驗：本試驗於處理組提供含毒食餌棉片與含水棉片，放置含水棉片是爲了模擬自然環境，以免高估含毒食餌棉片的致死效果。試驗時上述農藥個別測試，於處理組的籠內分別放入 1/100、1/200、1/500、1/1000 與 1/2000 (v/v) 等不同調配比例的含毒食餌棉片與含水棉片各一，對照組的籠內僅放入無毒食餌棉片，試驗設計採完全逢機設計 (CRD)，每處理與對照組各 3 重複，試驗開始後第 24、48 與 72 h 調查並紀錄籠內死亡蟲數與存活蟲數。

選擇性試驗：本試驗於處理組同時提供含毒食餌棉片及無毒食餌棉片，以供瓜實蠅選擇。挑選非選擇性試驗中經 72 h 處理後之平均累積死亡率大於 80% 的藥劑調配比例進行選擇性試驗，試驗時於處理組籠內分別放入經篩選後不同比例的含毒食餌棉片與無毒食餌棉片各一，對照組籠內僅放入無毒食餌棉片，試驗設計採完全逢機設計 (complete randomized design; CRD)，每處理與對照組各 3 重複，試驗開始後第 24、48 與 72 h 調查並紀錄籠內死亡蟲數與存活蟲數。

殘效試驗：挑選選擇性試驗中經 72 h 處理後之平均累積死亡率大於 80% 的藥劑調配比例進行殘效試驗，試驗時在第 0、1、2 及 3 wk 於處理組籠內放入同一片含毒食餌棉片，而每次皆置入新鮮的無毒食餌棉片，對照組籠內皆僅放入新鮮無毒食餌棉片，試驗設計採完全逢機設計 (CRD)，每處理與對照組各 3 重複，每週皆調查 72 h 後籠內死亡蟲數與存活蟲數。

資料分析

各組數據先經角度轉換 (angular transformation, arcsin $\sqrt{P}$)，再利用 SAS/EG 套裝統計分析軟體進行變方分析 (analysis of variance; ANOVA)，若有達顯著性差異，則採用 Fisher 的最小顯著差異性測驗 (Fisher's least significance difference test; LSD test) 以 5% 水準來檢測處理間之差異顯著性。

結果

非選擇性試驗

將瓜實蠅置於沒有無毒食餌棉片可供選擇的情況下進行試驗，瓜實蠅在取食後的累積死亡率隨著所暴露藥劑的濃度及時間增加而升高，依藥劑種類的不同，不同濃度所造成的瓜實蠅累積死亡率差異顯著性亦有不同 (表 1)。所有供試藥劑中，以添加 44% 大滅松乳劑的食餌對瓜實蠅的毒殺效果最好，在濃度 1/2000 時取食 24 h 後的死亡率達 84.7%，48 h 後達 98.0%。其次是添加 50% 芬殺松乳劑的食餌，在濃度 1/2000 時取食 24 h 後的死亡率僅 38.2%，但在 72 h 後達 85.1%。添加 40.8% 陶斯松乳劑的食餌在濃度 1/500 以上時 48 h 後的累積死亡率皆大於 90%。添加 50% 賽達松乳劑 50% 普硫松乳劑的食餌在濃度 1/500 以上時 72 h 後累積死亡率皆大於 80%。濃度 1/100 的 50% 撲滅松乳劑與 50% 馬拉松乳劑則僅在 72 h 後的累積死亡率大於 80%。推薦於可施用於瓜園的防治害蟲的 43% 佈飛松乳劑添加於餌劑之不同濃度對的累積死亡率皆未超過 80%。上述對瓜實蠅毒殺率達 80% 以上的農藥與濃度皆被選以進一步進行選擇性試驗。

選擇性試驗

同時提供瓜實蠅含毒與無毒食餌供其選擇，試驗結果顯示含大滅松與芬殺松的食餌在濃度 1/2000 時仍然對瓜實蠅具有較好的毒殺效果 (表 2)，含大滅松的食餌在 48 h 後對瓜實蠅的累積死亡率超過 80%，其次含芬殺松的食餌則在 72 h 後對瓜實蠅的累積死亡率超過 80%。含陶斯松的食餌在濃度 1/500 以上時在 24 h 雖然平均致死率低於 40%，但在 72 h 後的累積死亡率仍超過 80%。濃度 1/100 的含賽達松與普硫松食餌在 72 h 後的累積死亡率超過 80%。瓜實蠅推薦用藥馬拉松在濃度 1/100

表 1.　有機磷殺蟲劑與食餌以不同比例混合於非選擇試驗中對瓜實蠅之平均死亡率。

Table 1. Mean mortality of *Bactrocera cucurbitae* exposed to food baits incorporated with different rates of organophosphates in non-choice test.

Insecticide	Concentration (v/v)	% Cumulative mortality (Mean ± SD)[z]		
		24 h[y]	48 h	72 h
Chlorpyrifos 40.8% EC	1/100	74.1 ± 11.1 a	96.3 ± 3.0 a	99.5 ± 0.9 a
	1/200	62.9 ± 6.1 a	90.0 ± 6.8 a	98.5 ± 1.5 ab
	1/500	61.5 ± 11.8 a	92.6 ± 4.8 a	96.3 ± 0.9 b
	1/1000	25.1 ± 9.6 b	53.4 ± 5.8 b	75.0 ± 4.8 c
	1/2000	19.3 ± 6.0 b	36.9 ± 5.3 c	55.7 ± 7.1 d
CK		0.6 ± 1.0 c	2.6 ± 1.0 d	2.6 ± 1.0 e
Dimethoate 44% EC	1/100	82.7 ± 7.9 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	1/200	89.0 ± 8.0 a	97.9 ± 1.8 a	100.0 ± 0.0 a
	1/500	91.8 ± 5.7 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	1/1000	86.9 ± 1.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	1/2000	84.7 ± 2.9 a	98.0 ± 2.4 a	99.6 ± 0.8 a
CK		1.7 ± 3.0 b	2.3 ± 4.0 b	2.9 ± 5.0 b
Fenitrothion 50% EC	1/100	33.3 ± 8.5 a	68.9 ± 7.6 a	80.9 ± 2.9 a
	1/200	13.8 ± 3.1 b	47.2 ± 10.2 b	71.8 ± 2.4 a
	1/500	17.3 ± 6.3 b	35.9 ± 7.6 bc	49.5 ± 9.9 b
	1/1000	8.7 ± 7.7 bc	29.6 ± 8.2 c	47.0 ± 5.6 b
	1/2000	4.3 ± 2.5 c	7.0 ± 2.6 d	9.7 ± 3.0 c
CK		0.0 ± 0.0 d	0.0 ± 0.0 e	2.2 ± 1.9 d
Fenthion 50% EC	1/100	79.2 ± 2.3 a	99.5 ± 0.9 a	100.0 ± 0.0 a
	1/200	57.3 ± 13.2 b	88.5 ± 1.4 b	96.0 ± 2.4 b
	1/500	68.3 ± 5.0 ab	89.4 ± 1.1 b	96.8 ± 1.6 b
	1/1000	61.9 ± 5.4 b	80.8 ± 4.5 b	89.9 ± 8.0 cb
	1/2000	38.2 ± 11.8 c	56.9 ± 7.7 c	85.1 ± 3.0 c
CK		1.1 ± 0.9 d	2.7 ± 2.4 d	4.2 ± 3.9 d
Malathion 50% EC	1/100	41.7 ± 6.6 a	77.0 ± 8.9 a	87.0 ± 5.0 a
	1/200	28.0 ± 8.9 b	60.4 ± 9.9 b	78.8 ± 7.6 a
	1/500	9.2 ± 5.4 c	19.4 ± 11.6 c	38.5 ± 13.6 b
	1/1000	7.1 ± 3.6 c	12.7 ± 4.3 c	15.8 ± 2.6 c
	1/2000	4.2 ± 1.3 c	8.9 ± 4.3 c	14.0 ± 4.4 c
CK		0.5 ± 0.9 d	1.6 ± 0.1 d	3.3 ± 2.8 d
Phenthoate 50% EC	1/100	78.8 ± 17.9 a	92.5 ± 3.8 a	94.5 ± 4.1 a
	1/200	58.7 ± 14.6 b	84.2 ± 7.6 ab	96.4 ± 1.1 a
	1/500	52.8 ± 10.4 b	75.8 ± 9.3 b	83.2 ± 4.3 b
	1/1000	27.5 ± 4.5 c	51.8 ± 5.4 c	58.1 ± 6.0 c
	1/2000	19.1 ± 6.1 c	27.0 ± 8.7 d	41.3 ± 3.6 d
CK		0.0 ± 0.0 d	0.0 ± 0.0 e	0.5 ± 0.9 e

表 1.　有機磷殺蟲劑與食餌以不同比例混合於非選擇試驗中對瓜實蠅之平均死亡率 (續)。

Table 1.　Mean mortality of *Bactrocera cucurbitae* exposed to food baits incorporated with different rates of organophosphates in non-choice test. (continued)

Insecticide	Concentration (v/v)	% Cumulative mortality (Mean ± SD)[z]		
		24 h[y]	48 h	72 h
Profenofos 43% EC	1/100	38.2 ± 7.7 a	48.4 ± 1.4 a	67.7 ± 8.3 a
	1/200	29.5 ± 8.0 a	42.3 ± 5.5 a	60.6 ± 1.9 a
	1/500	37.2 ± 5.2 a	49.0 ± 9.3 a	59.8 ± 6.6 a
	1/1000	33.8 ± 8.5 b	36.9 ± 10.0 b	42.9 ± 7.6 b
	1/2000	16.2 ± 3.4 c	19.4 ± 4.3 c	25.7 ± 5.7 c
CK		0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
Prothiofos 50% EC	1/100	69.9 ± 8.4 a	93.9 ± 5.4 a	98.0 ± 1.8 a
	1/200	39.5 ± 9.3 b	84.1 ± 5.1 b	95.3 ± 1.8 a
	1/500	31.7 ± 9.2 b	71.6 ± 6.8 c	82.0 ± 6.0 b
	1/1000	8.1 ± 4.6 c	29.8 ± 6.8 d	50.4 ± 10.3 c
	1/2000	3.4 ± 0.1 c	10.7 ± 3.6 e	23.2 ± 5.3 d
CK		4.3 ± 1.1 c	7.3 ± 1.9 e	7.3 ± 1.9 e

[z] Means within different rates of an insecticides followed by the same letter are not significantly different at 5% level by Fisher's protected Least Significant Difference (LSD) test. Percentage data were subjected to arcsine-square-root transformation prior to analysis.

[y] Sampling time: hours after experiments initiated.

至 72 h 的累積死亡率僅達 67.1%。

殘效試驗

試驗結果顯示，以濃度 1/100 時含賽達松的食餌殘效性最佳 (表 3)。經過 1 wk 之後含毒食餌對瓜實蠅 72 h 平均死亡率超過 80% 僅剩下濃度 1/100 的陶斯松、大滅松、賽達松與普硫松，而經過 2 wk 之後，僅剩含賽達松的食餌對瓜實蠅 72 h 平均死亡率仍可維持在 80.8%。

討論

一個成功的誘殺劑最基本是要具備誘引能力與毒殺能力。瓜實蠅的誘殺劑可分為長距離誘殺雄蟲的克蠅香混合溶液與短距離同時誘殺雌雄蟲的食物誘引劑，食物誘引劑則包括蛋白質水解物與新鮮水果。在毒殺能力部份，有 58% 乃力松乳劑、50% 芬殺松乳劑、25% 馬拉松可濕性粉劑與 0.02% 賜諾殺濃餌劑。相關農藥添加於食物誘引劑中以防治瓜實蠅的研究多數是直接評估添加後的誘引效果 (Liu *et al*. 1997; Wang & Liu 2002) 或瓜果的受害率 (Fang & Chang 1986; Akhtaruzzaman *et al*. 2000)，對於著重評估篩選市售農藥添加於食物誘引劑後對瓜實蠅取食或接觸後之毒殺效果的研究則較少 (Lai & Yang 2012)。

本研究製成食餌所使用的酵母水解物於單獨使用 24 h 後對瓜實蠅雌蟲可達到 70% 以上的誘引能力，對雄蟲則更可達到 80% 以上 (Liu & Chen 1995)，因此該食餌除了提供瓜實蠅生存所需外，亦具有誘引效果。非選擇性試驗中瓜實蠅在僅有含水棉片與含毒食餌棉片下，可能造成瓜實蠅死亡的因子為取食含毒食餌或拒絕取食，兩者的差別在於有機磷類殺蟲劑是否對瓜實蠅產生忌避作用，若有忌避作用，未來有發展為忌避劑的潛力，若無忌避作用，則未來可以添加於誘引劑中進行毒殺。為模擬田間瓜實蠅的狀態，因此瓜實蠅在試驗前並未停止供應人工飼料，若瓜實蠅對含毒食餌出現忌避現象，但調查時間過短，可能無法顯現在致死效果上，因此將時間延長至 72 h。藉由此試驗結果排除致死率低於 80% 的藥劑與濃度組合，如 43% 佈飛松乳劑等 (表 1)，表示該藥劑與濃

表 2. 有機磷殺蟲劑與食餌以不同比例混合於選擇性試驗中對瓜實蠅之平均死亡率。

Table 2. Mean mortality of *Bactrocera cucurbitae* exposed to food baits incorporated with different rates of organophosphates in choice test.

Insecticide	Concentration (v/v)	% Cumulative mortality (Mean ± SD)[z]		
		24 h[y]	48 h	72 h
Chlorpyrifos 40.8% EC	1/100	39.7 ± 5.7 a	87.0 ± 10.0 a	93.5 ± 6.7 a
	1/200	32.3 ± 4.3 a	69.1 ± 8.1 b	85.7 ± 3.4 a
	1/500	36.3 ± 8.1 a	67.6 ± 4.9 b	82.8 ± 6.1 a
CK		2.0 ± 2.4 b	3.6 ± 3.6 c	5.1 ± 3.9 b
Dimethoate 44% EC	1/100	31.3 ± 4.0 b	93.9 ± 7.8 a	100.0 ± 0.0 a
	1/200	63.3 ± 9.7 a	90.4 ± 6.1 a	100.0 ± 0.0 a
	1/500	60.9 ± 5.8 a	84.7 ± 6.6 a	98.4 ± 2.7 a
	1/1000	62.5 ± 4.3 a	89.1 ± 7.1 a	98.5 ± 1.5 a
	1/2000	34.4 ± 10.6 b	84.5 ± 8.4 a	98.1 ± 1.7 a
CK		2.3 ± 2.6 c	3.4 ± 3.4 b	4.4 ± 2.7 b
Fenitrothion 50% EC	1/100	19.7 ± 7.1 a	43.7 ± 9.8 a	54.1 ± 0.8 a
CK		1.0 ± 1.7 b	2.5 ± 0.8 b	3.1 ± 0.2 b
Fenthion 50% EC	1/100	74.6 ± 2.7 a	95.9 ± 0.8 a	100.0 ± 0.0 a
	1/200	58.8 ± 11.7 b	82.9 ± 4.9 b	94.8 ± 1.7 b
	1/500	43.2 ± 8.3 c	80.6 ± 7.8 b	92.0 ± 5.6 b
	1/1000	46.6 ± 7.7 bc	66.7 ± 8.1 c	89.5 ± 3.4 b
	1/2000	39.0 ± 3.2 c	68.6 ± 6.4 c	89.7 ± 1.4 b
CK		2.2 ± 2.7 d	3.3 ± 3.1 d	4.3 ± 2.2 c
Malathion 50% EC	1/100	17.0 ± 6.1 a	44.3 ± 6.4 a	67.1 ± 5.0 a
	1/200	11.6 ± 8.3 a	32.0 ± 16.2 a	47.5 ± 8.7 b
CK		1.6 ± 1.6 b	3.6 ± 1.1 b	5.2 ± 2.7 c
Phenthoate 50% EC	1/100	69.2 ± 9.2 a	81.6 ± 8.1 a	92.7 ± 3.5 a
	1/200	25.3 ± 12.6 b	49.1 ± 4.8 b	58.9 ± 1.4 b
	1/500	25.9 ± 5.4 b	44.3 ± 4.5 b	58.9 ± 1.4 b
CK		0.0 ± 0.0 c	1.6 ± 0.0 d	2.6 ± 1.0 c
Prothiofos 50% EC	1/100	12.6 ± 5.8 b	44.7 ± 9.9 a	82.4 ± 4.7 a
	1/200	23.5 ± 8.2 a	48.6 ± 8.1 a	66.1 ± 2.8 b
	1/500	6.4 ± 1.8 bc	20.4 ± 6.5 b	41.7 ± 3.7 c
CK		4.3 ± 1.2 c	7.4 ± 2.0 c	7.4 ± 2.0 d

[z] Means within different rates of an insecticides followed by the same letter are not significantly different at 5% level by Fisher's protected Least Significant Difference (LSD) test. Percentage data were subjected to arcsine-square-root transformation prior to analysis.

[y] Sampling time: hours after experiments initiated.

度組合對瓜實蠅佈論在毒殺或忌避上的效果皆不佳，而將瓜實蠅死亡率超過 80% 的藥劑與濃度組合再以選擇性試驗進一步釐清其主要致死因子。

選擇性試驗中評估同時放置含毒食餌棉片與無毒食餌棉片對瓜實蠅的致死效果，若該藥劑對瓜實蠅具有忌避作用，則在提供無毒食餌棉片的情況下，瓜實蠅的死亡率應該極低。比較表 1 與表 2，瓜實蠅在相同藥劑、濃度與調查時間條件下，選擇性試驗的平均死亡率皆低於非選擇性試驗。除了含 50% 芬殺松乳劑的食餌外，其他藥劑的食餌在選擇性試驗中 48 h

表 3. 陶斯松、大滅松、芬殺松、賽達松或普硫松與食餌混合後之殘效對瓜實蠅 72 h 之平均死亡率。

Table 3. Residual effects of food baits incorporated with chlorpyrifos, dimethoate, fenthion, phenthoate or prothiofos on mean mortality of *Bactrocera cucurbitae* at 72 h after treatment.

Insecticide	Concentration (v/v)	Mean mortality (%)[z]			
		0 wk[y]	1 wk	2 wk	3 wk
Chlorpyrifos 40.8% EC	1/100	93.5 ± 6.7 bc	85.0 ± 3.8 ab	16.5 ± 8.6 e	-
	1/200	86.2 ± 3.2 de	68.7 ± 7.1 de	9.3 ± 2.3 ef	-
	1/500	84.1 ± 4.2 e	20.8 ± 2.9 i	5.0 ± 6.2 gh	-
Dimethoate 44% EC	1/100	100.0 ± 0.0 a	83.8 ± 2.6 ab	57.3 ± 7.1 b	26.8 ± 6.5 b
	1/200	100.0 ± 0.0 a	78.5 ± 8.1 bc	47.0 ± 2.6 bc	22.2 ± 4.7 bc
	1/500	98.9 ± 1.8 a	71.1 ± 6.8 cd	37.7 ± 2.4 cd	15.9 ± 1.2 cd
	1/1000	99.0 ± 0.8 a	63.6 ± 6.9 def	36.3 ± 7.9 cd	13.5 ± 1.3 d
	1/2000	98.6 ± 1.4 ab	59.9 ± 3.4 ef	29.7 ± 9.6 d	12.2 ± 0.6 d
Fenthion 50% EC	1/100	100.0 ± 0.0 a	59.5 ± 8.3 ef	7.9 ± 4.3 fg	-
	1/200	95.3 ± 1.4 bc	55.8 ± 3.5 fg	7.5 ± 2.8 fg	-
	1/500	92.9 ± 4.7 cd	48.4 ± 4.0 g	7.9 ± 1.6 efg	-
	1/1000	90.4 ± 2.6 cd	36.4 ± 0.8 h	6.7 ± 3.3 fgh	-
	1/2000	89.3 ± 0.8 cde	36.0 ± 2.4 h	5.8 ± 1.6 fgh	-
Phenthoate 50% EC	1/100	93.2 ± 2.7 cd	90.0 ± 1.4 a	80.8 ± 5.2 a	57.0 ± 9.4 a
Prothiofos 50% EC	1/100	82.6 ± 4.8 e	81.3 ± 6.2 b	55.3 ± 7.7 b	16.7 ± 4.5 cd
CK		0.5 ± 0.9 a	1.5 ± 1.5 j	2.1 ± 0.9 h	6.1 ± 1.5 e

[z] Means followed by the same letter are not significantly different at 5% level by Fisher's protected Least Significant Difference (LSD) test. Percentage data were subjected to arcsine-square-root transformation prior to analysis.
[y] Data indicate mean and its standard deviation of 3 replicates. Exposure time of food baits in the greenhouse.

後的平均死亡率與在非選擇試驗中 24 h 後的平均死亡率相近。這可能與選擇性試驗中提供無毒食餌棉片有關，瓜實蠅因可選擇無毒食餌而降低與含毒食餌接觸或取食的機率，造成對瓜實蠅的致死效果降低。依選擇性試驗推測供試有機磷類殺蟲劑添加於食餌中並不會對瓜實蠅有忌避作用，而其主要的致死原因是取食或接觸添加有機磷類殺蟲劑的食餌。此試驗中含 44% 大滅松乳劑的食餌在濃度 1/100 時 24 h 後對瓜實蠅的平均死亡率僅 31.3%，雖然 48 h 後亦達 93.9%，此一現象是否表示高濃度的大滅松可能會對瓜實蠅接觸或取食食餌行爲產生影響，則需再提高濃度進行試驗才可得知。

添加於餌劑內的殺蟲劑除了評估其毒殺效果外，其殘效持續時間爲另一個評估重點。以食物餌劑而言，影響殘效毒殺效果的因子除了藥劑本身的藥效消退之外，發霉所造成食餌棉片被遮蔽，導致瓜實蠅無法取食到含毒食餌，進而影響毒殺效果亦是重要因子。由於食餌在配置時並無添加防腐劑或殺真菌劑，雖然成品農藥中或許有添加抗菌劑，但是將其添加於食餌後，抗菌劑的效果亦受到某些程度影響，因此在殘效試驗中吸附含農藥食餌的棉片多數在 1 wk 後開始發霉，可能間接影響瓜實蠅取食含毒食餌的毒殺效果，因此未來在含毒食餌研究上應考慮防腐劑或殺真菌劑的添加，以降低發霉對瓜實蠅誘殺效果的影響。

目前防治瓜實蠅是推薦以 25% 馬拉松可濕性粉劑稀釋 400 倍添加蛋白質水解物點噴植株 (Fei *et al*. 2010)，避免農民購買防治藥劑的困擾，在本試驗中則是選用可供防治對象較廣的 50% 馬拉松乳劑添加於食餌。若以非選擇性試驗結果推測推薦藥劑濃度對瓜實蠅的毒殺效果，其馬拉松有效濃度爲 0.0625% 約是在濃度 1/500 至 1/1000 之間，對瓜實蠅在 24 h 後的平均致死率約 7 至 9%，而在 72 h 後也僅達 15–38%，恐已難符合農民防治瓜實蠅上的需求，且 43% 佈飛松乳劑對瓜實蠅的防治

效果亦不佳。因此未來若能在田間證實市售的陶斯松、大滅松、芬殺松、賽達松或普硫松添加於食物誘引劑對瓜實蠅的誘引能力不造成干擾，則自製成餌片或噴施在園區周圍的雜木上，作為防治的選擇之一。另一方面，將陶斯松、大滅松、賽達松或普硫松添加於克蠅或克蠅香內，與目前推薦的 50% 芬殺松乳劑作毒效比較，亦是未來可考慮的研究主題。目前國內推薦於克蠅與克蠅香內以 10：1 的比例添加 50% 芬殺松乳劑 (Fei *et al*. 2010)，此添加比例之芬殺松的濃度約為 5%，而本研究中芬殺松的濃度僅 250 ppm，即可對瓜實蠅產生接近 90% 毒殺效果，未來若能證實其添加於克蠅或克蠅香內的殘效能達 45 d，即可符合國內多數瓜類栽培期間防治瓜實蠅之所需。

綜合本試驗的結果，供試之有機磷殺蟲劑中，陶斯松、大滅松、芬殺松、賽達松與普硫松添加於食餌中對瓜實蠅具有較佳的毒殺效果，應用餌劑誘殺除了能降低農民使用有機磷類殺蟲劑直接噴施在瓜果上防治瓜實蠅所造成過量農藥殘留的風險外，更能降低有機磷殺蟲劑對人體、生態與環境的傷害。未來若研發出適當的餌劑，可以進一步評估其在田間防治瓜實蠅的效果。

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Lethal Effects of Organophosphates Mixed with Adult Food against the Melon Fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

Hsin-Shun Lai[1,*]

Abstract

Lai, H. S. 2013. Lethal effects of organophosphates mixed with adult food against the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). J. Taiwan Agric. Res. 62(4):372–381.

The study was conducted to evaluate effects of five ratios of eight organophosphates in adult food on the mortality of the melon fly, *Bactrocera cucurbitae* (Coquillett). Higher ratios of insecticides resulted in increasing melon fly mortalities. As there were no non-insecticide food baits provided for melon fly's choice (no-choice test), baits mixed with dimethoate caused higher mortalities compared to other insecticides tested on the melon flies. Dimethoate baits at a concentration of 1/2000 (v/v) killed approximately 85% of the flies tested at 24 h, while it caused 98% mortality at 48 h. On the other hand, the profenofos bait was least effective to the melon fly. In a non-insecticide bait provided together with an insecticide bait to the melon flies for choice (choice test), dimethoate bait still caused the highest mortalities to the flies tested, while fenitrothion and malathion baits resulted in the lowest ones. Melon fly mortalities were higher in the no-choice tests than the choice. Phenthoate bait maintained the highest residual toxicity among all of the baits tested. This bait at a concentration of 1/100 after two weeks of exposure in the greenhouse still caused over 80% of mortalities. Collectively, this study showed that chlorpyrifos, dimethoate, fenthion, phenthoate and prothiofos elicited high toxicity to the melon fly while incorporated into the adult food, and were potential to be considered as the insecticide additives in melon fly baits.

Key words: *Bactrocera cucurbitae*, Organophosphates, Baits.

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不同果肉類型桃果實採收後硬度與果膠質之變化

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摘要

羅淑卿、賴盈璋、歐錫坤。2013。不同果肉類型桃果實採收後硬度與果膠質之變化。台灣農業研究 62(4):382–388。

果膠分子間之鍵結被認為是影響蔬果質地變化之重要因素。為探討桃不同果肉類型採收後，果實軟化與果膠層化學鍵結之間的相關變化，選擇果肉溶質型之 '春蜜' 及 '台農甜蜜' 和硬肉類型之 '鶯歌桃'，測定採後 12 d 內之果實硬度、果膠酯化度 (degree of esterification; DE)、結合態鈣 (bound calcium) 及非甲氧基酯鍵 (non-methoxy ester linkage; NME) 等含量變化。結果顯示，採後第 3 d，'春蜜'、'台農甜蜜' 硬度急速下降，低於 '鶯歌桃' 3 倍，由硬度差異變化顯示其果肉特性。三品種桃子隨著貯藏時間增長，總果膠含量及 DE 值隨之下降，果膠含量由原每克酒精不溶物 (alcohol insoluble solids; AIS) 含 513 mg AGA (anhydrogalacturonic acid) 降至 350 mg AGA，而 DE 值由 45% 下降至 29%，以 '春蜜' 降幅最大。對硬度有正面貢獻之結合態鈣含量，則隨貯藏時間增長而呈現先增後降之變化，以貯藏第 3 d 之 '鶯歌桃' 為最高，達 6.7 mg $g^{-1}AIS^{-1}$，隨貯藏時間增長，三品種桃皆降至 2 mg $g^{-1}AIS^{-1}$ 左右。另一對硬度有正面貢獻之 NME，其含量及變化則依各果肉類型而異，在相同類型之桃子中，總酯鍵、NME 之變化趨勢相同，'春蜜' 和 '台農甜蜜' 桃隨貯藏時間之增長有先增後降之趨勢，而 '鶯歌桃' 則隨貯藏時間延長而增加。若能進一步建立桃不同類型採後初期果膠分子與果膠酵素活性變化之相關性，可作為未來選用貯運、加工及保鮮方式之重要參考依據。

關鍵詞：桃子、果膠、酯化度、結合態鈣、非甲氧基酯鍵。

前言

果膠質 (pectin) 賦予果實食用時之脆度、黏度或滑順口感，存在所有高等植物細胞與細胞間之中膠層內，就如同磚塊與磚塊間的水泥，作為細胞間的黏結物質。果膠本身為一種高分子量長鏈聚合體，組成單體為 α-1,4-D 半乳糖醛酸 (D-galacturonic acid)。影響蔬果質地軟或硬之重要因素，被認為是果膠質在後熟或烹煮過程中，產生酵素活化、降解、流失或許多化學結構之改變 (Ghasemlou *et al*. 2012; Missang *et al*. 2012; Yadav *et al*. 2012; Itamura *et al*. 2013)。

桃生產市場逐漸全球化，桃果實質地乃決定消費者接受與否的重要因素之一，因此控制完熟過程及防止果實品質劣變為桃樹生產事業的主要重點。台灣平地、淺山坡地所種植的低需冷量桃樹品種，其果肉質地涵蓋果肉溶質類型 (melting flesh) 及如石頭般堅硬之硬肉桃類型 (stony hard)。本研究選用平地低需冷量桃樹 '春蜜'、'台農甜蜜' (溶質類型) 與 '鶯歌桃' (硬肉類型) 等 3 個栽培種為試驗材料，探討果實貯藏過程中，果肉質地與果膠鍵結之化學變化，以瞭解台灣不同果肉類型桃，採收後質地變化與果實生理之關係。此研究成果可供農民業者作為選種依據，或產業採收後保鮮處理及後續加工之研發應用。

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材料與方法

本試驗收集行政院農業委員會農業試驗所種植 ‘春蜜’、‘台農甜蜜’ (溶質類型) 與 ‘鶯歌桃’ (硬肉類型) 等 3 個栽培種爲供試材料，進行硬度、總果膠含量、果膠 DE 值 (degree of esterification)、結合態鈣離子、果膠分子總酯鍵含量及 NME (non-methoxy ester linkage) 含量的測定。硬度測量依果實實際硬度的高低選取硬度計的鑽頭 [Fruit Pressure Tester, model FT 327 (1.36–12.25 kg), Facchini, 48011 Alfonsine, Italy]。三種桃果實重量分別爲 ‘春蜜’ 132.8 g、‘台農甜蜜’ 114.1 g、‘鶯歌桃’ 151.8 g。果實達 8 分熟以上的成熟度採收，在室溫下 (23–27℃) 依 0、3、6、9、12 d 取樣一次，每次取樣 5 個果實，3 重複，計 15 個果實。

每一個果實表面的赤道部分切除直徑 1–2 cm 的果皮，再以硬度計測定之。在果實測定點的正對面，選定第二點，再測讀數，並取其平均值爲硬度 (Ou *et al*. 2006)。桃子果肉依 Hou & Chang (1996) 所提方法製成酒精不溶物 (AIS) 以進行果膠鍵結特性之分析測定，並繪製標準曲線 (圖 1)。AIS 的製備爲桃果肉加入 95% 乙醇使乙醇最終濃度爲 80%，均質過濾後，殘餘再以 80% 乙醇均質過濾至濾液檢測不出醣爲止，再乾燥磨粉備用。果膠含量之測定爲將各種 AIS 以濃硫酸液化、稀釋，再以 m-hydroxydiphenyl 呈色測定 520 nm 吸光值，對照半乳糖醛酸溶液 (0–150 μg mL^{-1}) 依同法所製的標準曲線定量，定量結果以 anhydrogalacturonic acid (AGA) 含量表示。果膠甲酯化程度之測定以氫氧化鉀皂化 AIS 後，調整 pH 至 7.5 並定容最終濃度爲 25–200 μg AGA mL^{-1}，加入 alcohol oxidase (1 unit mL^{-1}) 反應並以呈色劑 (0.02 M 2,4-pentanedione/2.0 M acetate/0.05 M acetic acid) 呈色，測定 412 nm 波長之吸光。所得結果對照甲醇溶液所製成之標準曲線定量，將甲醇含量乘以 31 再除以 32，再除以總果膠含量，即得甲氧基含量百分率。將甲氧基含量百分率除以 16.32% (理論上最高甲氧基含量) 再乘以 100，即得甲酯化程度百分率 (%)。

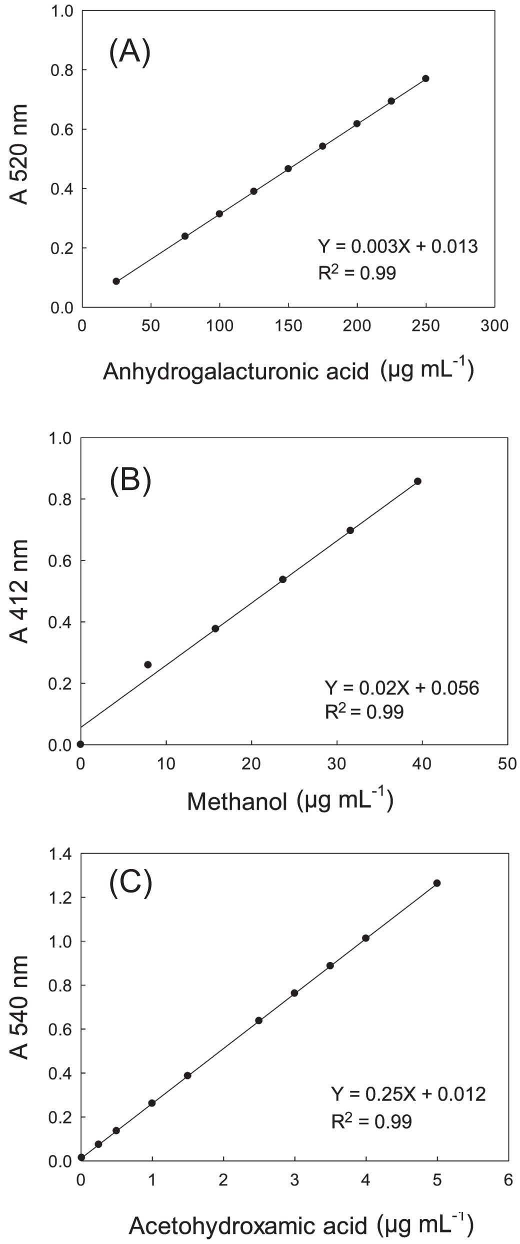

圖 1. 無水半乳糖醛酸 (anhydrogalacturonic acid) (A)；甲醇 (B)；及醋羥胺酸 (acetohydroxamic acid) (C) 之標準品檢量線。

Fig. 1. Standard curves of anhydrogalacturonic acid (A), methanol (B), and acetohydroxamic acid (C).

AIS 中鈣離子的定量以濃硫酸炭化 AIS 後，進行溼式消化分解 (wet digestion) 並以蒸餾水定容後，以感應耦合電漿光譜分析儀

(ICP) 測定鈣離子含量。AIS 中總酯鍵含量之測定爲 AIS 以 0.5 M 鹼性羥基胺 (hydroxylamine) 溶液反應後，以鹽酸調整反應液 pH 約 6.0 以終止反應，經適當稀釋後，加入 12% HCl 及 5% 氯化鐵溶液呈色，測定 540 nm 吸光值，依同法以羥柉醋酸 (acetohydroxamic acid, $CH_3CONHOH$) 製作標準曲線，結果以 μeq $CH_3CONHOH$ mg^{-1} AIS^{-1} 表示。反應後殘渣，利用 2 M 三氟醋酸於 120℃ 高溫水解，過濾後加入 sodium acetate 定量，再加入 0.2 M 之磷酸鹽緩衝液 (pH 3.9)，再以碘液 (0.5 mL) 將羥基胺氧化成亞硝酸後，加入 sodium arsenite (1.5% w v^{-1}) 及 naphthylethylenediamine (1.5% w v^{-1}) 呈色，即可測定 543 nm 波長之吸光值，依同法以羥基胺製作標準曲線以定量。將上述濾液中所得數值加上殘渣內所得數值，即爲 AIS 中總酯鍵含量，以 μeq $CH_3CONHOH$ mg^{-1} AIS^{-1} 表示。AIS 中非甲氧酯鍵含量之求算：由所得總酯鍵含量扣掉 AIS 中果膠的甲氧鍵含量 (利用 alcohol oxidase 法測定者)，即得非甲氧酯鍵含量，以 μeq $CH_3CONHOH$ mg^{-1} AIS^{-1} 表示。

結果與討論

剛採收的桃子不論何種類型，硬度皆於 15 kg cm^{-2} 以上，以 ‘鶯歌桃’ 爲最硬，達 18 kg cm^{-2} (圖 2)。隨貯藏時間拉長，各類桃硬度值皆隨之急速下降，‘台農甜蜜’ 及 ‘春蜜’ 桃於貯藏第 3 d，硬度即迅速降至 4.5 kg cm^{-2}，果實質地外觀已呈現軟化多汁現象，於 6 d 後硬度已降至一定值。‘鶯歌桃’ 直至第 9 d 才降至 5.0 kg cm^{-2}，貯藏全程時間硬度皆高於 ‘台農甜蜜’ 及 ‘春蜜桃’。硬度測定結果顯示二類桃硬度值下降程度差異極大，‘台農甜蜜’ 及 ‘春蜜’ 桃下降速度顯然快過 ‘鶯歌桃’。採收後第 9 d 以之 ‘台農甜蜜’ 及 ‘春蜜’ 桃外觀已呈現軟化爛熟之現象，因此各項測定值皆未探討 9 d 後之情形。

剛採收之各品種桃子總果膠量以 ‘鶯歌桃’ 爲最高 (圖 3A)，爲 500 mg AGA g^{-1}AIS^{-1}，‘春蜜’ 最低，爲 470 mg AGA g^{-1}AIS^{-1}。隨貯藏時間的增長，‘春蜜’、‘台農甜蜜’ 及 ‘鶯歌桃’ 的

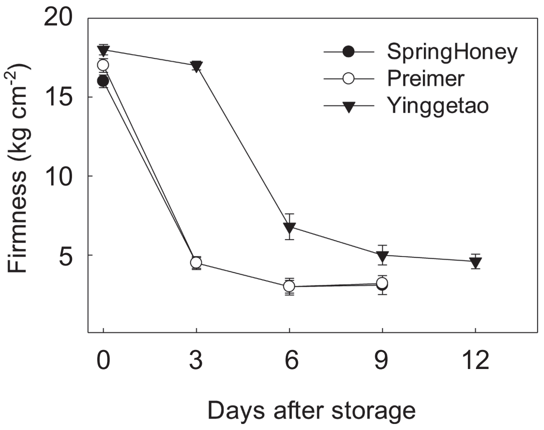

圖 2. 不同果肉類型的桃於貯藏期間硬度值之變化。

Fig. 2. Changes in firmness of different peach flesh types stored at 25℃ ± 2℃. The vertical bars represent the standard deviation (SD) of 3 replicates.

總果膠含量逐漸下降，降低幅度最大者爲 ‘台農甜蜜’，減少量爲 140 mg AGA g^{-1}AIS^{-1}。‘鶯歌桃’ 的總果膠量變化較緩和，貯藏至第 12 d 仍有 439 mg AGA g^{-1}AIS^{-1}。剛採收之各品種桃子果膠 DE 值以 ‘春蜜’ 最高 (圖 3B)，爲 45%。隨貯藏時間增長，三種桃子 DE 值皆隨之下降，貯藏至第 9 d 皆降至 30% 以下，下降幅度以 ‘春蜜’ 爲最大 (16%)。

剛採收之各品種桃子結合態鈣含量以 ‘鶯歌桃’ 爲最高 (圖 3C)，含量爲 5.7 mg g^{-1}AIS^{-1}。隨貯藏期間增長，‘鶯歌桃’ 和 ‘台農甜蜜’ 會隨時間增長而結合態鈣含量增加，於貯藏第 3 d，以 ‘鶯歌桃’ 爲最高，達 6.7 mg g^{-1}AI^{-1}。第 3 d 後，結合態鈣含量會隨貯藏時間增長而快速降低，三種桃子於貯藏第 6 d 後皆降至 2 mg g^{-1} AIS^{-1} 以下。

剛採收之各品種桃子總酯鍵含量及變化依各品種而異 (圖 3D)。‘春蜜’ 和 ‘台農甜蜜’ 隨貯藏時間之增長，總酯鍵含量有先增後降之趨勢，以第 3 d ‘台農甜蜜’ 爲最高，達 1.0 μeq $CH_3CONHOH$ mg^{-1} AIS^{-1}。硬肉桃類之 ‘鶯歌桃’ 總酯鍵含量則貯藏時間之增長而增加，第 12 d 達 0.6 μeq $CH_3CONHOH$ mg^{-1} AIS^{-1}，結果可見變化趨勢於同類型之桃子如 ‘春蜜’ 和 ‘台農甜蜜’ 則相同。剛採收之各品種桃子非甲氧基酯鍵含量變化趨勢同總酯鍵，‘春蜜’ 和 ‘台

農甜蜜' 含量隨貯藏時間之增長而呈現先增後降之趨勢，但 '鶯歌桃' 則隨貯藏時間之增長而增加 (圖 3E)。由此可見，變化趨勢在同類型之桃子如 '春蜜' 和 '台農甜蜜' 爲相同。

桃子剛採收時硬度差異並不大，一般只憑外觀並無法分辨爲果肉溶質類型或果肉硬肉類型。由於桃子有後熟行爲，在貯藏過程中，質地之崩解變化才顯現出果肉類型之不同。果膠分子結構變化爲影響蔬果質地軟或硬之重要因素，本研究試驗結果顯示於貯藏過程中，桃子之果膠分子呈現的變化及趨勢，印證文獻所提，不同類型桃果實採後硬度之變化與果膠分子結構改變之關係理論。桃子剛採收時，三品種桃子硬度相當，有最高的 DE 值。果膠分子之部分羧基與甲醇結合而成高酯化度之狀態，形成不溶於水之原果膠 (protopectin)，並和纖維素和半纖維素有較緊密之結合，隨著果實貯藏時間增長，發生後熟現象，原果膠會受原果膠酵素 (protopectinase)、多聚半乳醣醛酸酶 (polygalacturonase; PG) 等酵素作用水解爲水溶性之果膠酯酸 (pectinic acid)，果實因而軟化 (Sila *et al*. 2009)。若果膠酵素進一步將果膠長鏈分子水解爲單體 (monomer) 或短鏈成分，果膠和細胞間的吸附減弱，果實質地呈現

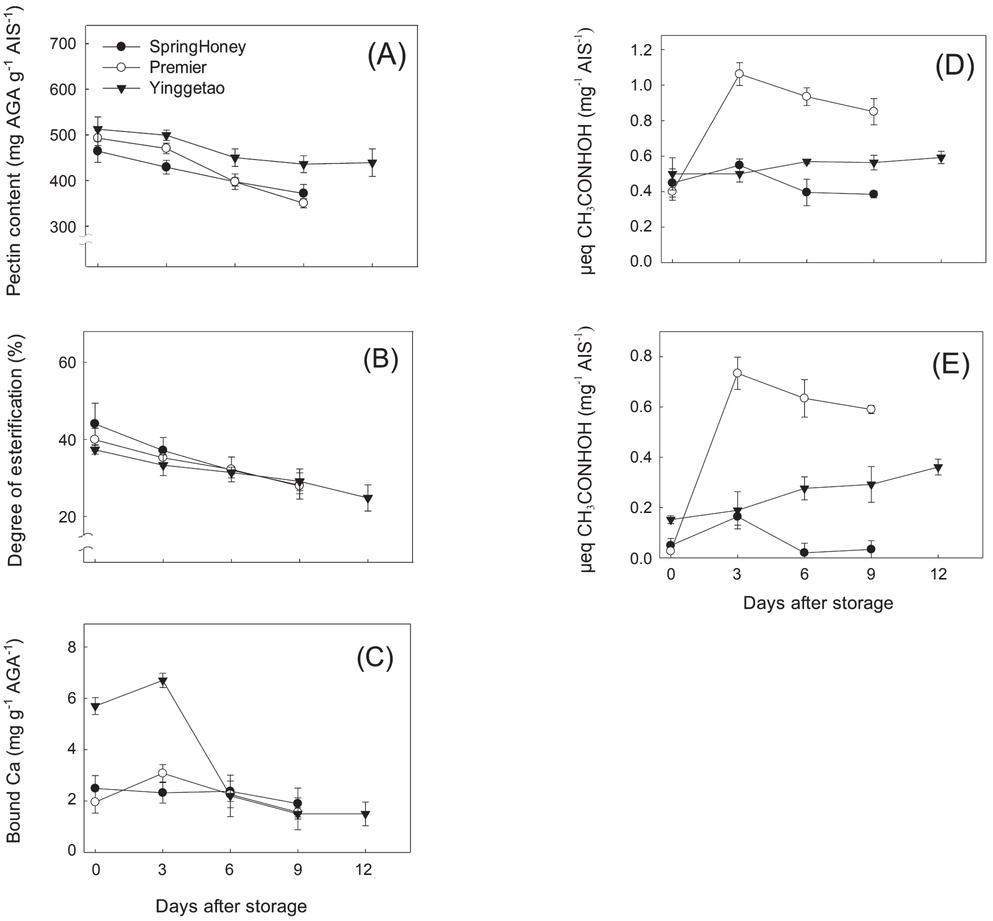

圖 3. 桃不同果肉類型貯藏期間總果膠含量 (A)；酯化度 (B)；結合態鈣含量 (C)；總酯鍵 (D) 及非甲氧基酯鍵含量 (E) 等變化。

Fig. 3. Changes in pectin content (A), degree of esterification (DE) (B), bound calcium content (C), total ester linkages (D), and non-methoxy ester linkages (NME) (E) of different peach flesh types stored at 25℃ ± 2℃. The vertical bars represent the standard deviation (SD) of 3 replicates.

軟爛過熟，造成硬度值下降 (Medina-Escobar *et al*. 1997)。總果膠量隨貯藏時間增加而下降，研判受水解作用後之果膠小分子，不會存留於 AIS 中，因此隨貯藏天數之增加，小分子增多流失也多，造成果實總果膠含量後熟初期高於後熟後期 (Shewfelt 1965; Magallhaes *et al*. 1996; Yadav *et al*. 2012)。

供試的 3 個桃子品種中，‘鶯歌桃’ 貯存 9 d 後的硬度值仍能大於果肉溶質類型之桃子。依 Brummell *et al*. (2004) 指出桃子兩個重要果膠酵素 exo-PG 及 endo-PG，於後熟初期皆存於各種不同類型之桃子。但於桃子後熟後期，可進一步將長鏈果膠分子切成單體之 endo-PG，只出現在溶質類型之桃子，所以果肉會呈現多汁、溶融狀，推測 ‘鶯歌桃’ 貯藏全程果實硬度皆大過溶質類型桃子是因未受 endo-PG 進一步作用或酵素活性較低所致。由於果膠裂解過程同時需要果膠甲酯酶 (pectinesterase; PE) 作用切去部分果膠分子之甲氧基，以降低果膠質 DE 值才易受 PG 進一步將果膠長鏈分子水解為單體 (Medina-Escobar *et al*. 1997; Brummell & Harpster 2001)。因此，隨著後熟時間增長，三品種桃子果膠 DE 值皆下降，不同類型之桃子本身有不同的 DE 值，但下降趨勢卻是相同。

酵素水解果膠分子對硬度屬於破壞性作用，但有些伴隨之化學變化是對硬度有建設性作用。依 Bartolome & Hoff (1972) 所提果實質地硬化反應機制，在 PE 酵素作用降低果膠 DE 值之同時，暴露出的游離羧基會和內源性的鈣離子形成鈣架橋結構，使其結合態鈣含量增加，鈣架橋結構對硬度有正面貢獻 (de Freitas *et al*. 2012; Peaucelle *et al*. 2012)。分析結合態鈣含量可推測鈣架橋結構生成情形，由結果可見硬肉類型之 ‘鶯歌桃’ 鈣含量較高於果肉溶質型桃，對照硬度維持情形，推測鈣架橋之生成對 ‘鶯歌桃’ 硬度之維持具正面貢獻。

另一對果肉組織硬度有正面貢獻的鍵結為非甲氧基酯鍵。受活化的 PE 酵素除了水解果膠分子之甲氧基外，亦催化了轉醯基化反應，在果膠分子和細胞壁組織多醣分子間形成非甲氧基酯鍵之新酯鍵 (Hou & Chang 1996; Wang *et al*. 2013)。由分析結果顯示，不同桃子總酯鍵和非甲氧基酯鍵含量隨後熟時間增長呈現不同消長趨勢，後熟初期轉醯化反應確實發生，但新酯鍵之生成時間與維持，在不同果肉類型之桃子變化不一。‘鶯歌桃’ 於貯藏後期，即使貯放至 12 d，組織質地只呈現韌化現象，而無變化成軟爛多汁。Lurie *et al*. (2003) 曾指出 80% 之水果貯存後，組織韌化非因其果膠質瓦解，而是去酯化所造成，因去酯化之同時又可能形成新酯鍵。由本研究結果分析推測，‘鶯歌桃’ 後熟時期組織不致像溶質型桃子般崩解，新酯鍵之形成具一定程度之貢獻。由於果肉溶質型之桃子採收後質地軟化相當快，組織及細胞間隔快速瓦解，促使 PE 和 PG 活性釋出，更加速果膠分子之水解，果膠分子內的破壞性大過於建設性，因此本研究未能觀察得鈣架橋結構、新酯鍵初形成時，是否對硬度有維持效果。若在果實後熟初期，採樣天數更密集，以偵測採後短時間內各項參數之變化情形，將可進一步確立鈣架橋結構、新酯鍵對硬度之正面貢獻相關性。未來進一步探討不同類型桃子之 PG 和 PE 活性差異，可作為維持桃子硬度之重要依據。此外，研究報告指出果膠具許多生理活性 (抗肥胖、抗糖尿病、抗膽結石和抗癌) (Jiang *et al*. 2012; Yang *et al*. 2013)，在國內養生保健的意識抬頭下，亦可作為桃果實的育種目標。

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Changes in Firmness and Pectin Properties of Different Peach Types during Ripening

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Abstract

Lo, S. C., Y. J. Lai, and S. K. Ou. 2013. Changes in firmness and pectin properties of different peach types during ripening. J. Taiwan Agric. Res. 62(4):382–388.

The interaction between pectin molecules affects the changes of texture in both fruit and vegetables. To investigate the linkage of firmness and pectin to texture changes in different flesh types of peach, traits of firmness, degree of esterification (DE), bound calcium content, and non-methoxy ester linkage (NME) content of melting flesh peach (‘Spring Honey’ and ‘Premier’) and stony hard peach (‘Yinggetao’) were analyzed during twelve-day ripening period after harvest. Results indicated that firmness of ‘Spring Honey’ and ‘Premier’ decreased rapidly and their values were three-fold lower than ‘Yinggetao’ in three days after harvest, suggesting a different flesh characteristic between these two types of peach. The total pectin content and DE value of all peach cultivars were decreased after harvest, from 513 to 350 mg anhydrogalacturonic acid g^{-1} alcohol insoluble $solids^{-1}$ in total pectin and from 45 to 29% in DE value, respectively. ‘Spring Honey’ showed the largest decreased. The bound calcium had a positive effect on firmness and was decreased with the increasing of ripening after rising in the early stage. ‘Yingtao’ contained highest bound calcium, 6.7 mg g^{-1} AIS^{-1}, at three days after harvest, but its content decreased during ripening, reached to about 2 mg g^{-1} AIS^{-1} at the end of experiment as other peach cultivars. NME also had a positive effect on firmness, whereas, different types of peach had vary contents and changing patterns during ripening period. The total ester linkages and NME followed the same changing pattern in the same peach type; ‘Spring Honey’ and ‘Premier’ were first increased and then decreased, while ‘Yingtao’ was increased throughout the storage. As a result, for establishing a better relationship between pectin molecules and pectinase activity for different types of peach during initial phase of storage after harvest, storing conditions and methods of processing and preservation should be taken into account altogether.

Key words: Peach, Pectin, Degree of esterification, Bound calcium, Non-methoxy ester linkage.

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一、 本刊為行政院農業委員會農業試驗所發行之學術性期刊 (季刊)，刊登國內、外農業試驗研究相關之研究稿件：

論　　說：由本所期刊編審會邀請專家撰寫。

研究報告：關於農業科學未曾發表之研究報告，按題目、作者、摘要、前言、材料與方法、結果、討論、結論 (一般略去，或併入討論)、誌謝 (可略去)、引用文獻等依序撰寫，表與圖附於其後，全文以不超過10印刷頁為原則。其中，'結果'與'討論'如有必要，可合併成'結果與討論'。

研究簡報：關於農業科學未曾發表之簡短原始研究報告，不須分章節撰寫(可參照研究報告次序)，但須附中英文摘要，全文以不超過4印刷頁為原則。

二、 稿件應以中文或英文撰寫，以文書軟體MS WORD®格式儲存成檔案 (檔案類型以Word 97-2003文件為佳)。文稿以A4大小 (長29.6公分，寬21公分) 兩倍行高 (double space) 繕打，加行號，12號字體，左邊對齊，版面應預留上下3公分，左右2公分之空間，並自第二頁起在右下角以阿拉伯數字註明頁數。撰寫時，中文以細明體、英文以Times New Roman字體打字；英文字距及句距皆一字節。

三、 投稿者在本刊線上投審系統首頁點選'投稿者區域'，在一般稿件提交區點選'作者提交新稿件'進入，確認及點選稿件已依照稿約撰寫無誤後，下一步即填寫稿件資料，務請填寫封面信函，再依序填寫作者資料、上傳檔案及確認提交。本刊線上投審系統僅接受一位通訊作者，稿件如有多位通訊作者，務請註明於封面信函中，否則不受理，本刊將於稿件接受刊登後逕予加註多位通訊作者。

四、 稿件格式：

題　　目：宜簡短，能充分反應文旨，並附簡題 (Running title) (中文12字；英文6字以內為原則)。英文題目除介系詞、連接詞等字外，每字第一字母應大寫。報告如有機關所屬編號，請列於誌謝。

作　　者：作者姓名置中，作者二人或二人以上時，以中文一全形空格間隔之；英文姓名則名在前姓氏在後，作者二人或二人以上時，以 " , " 相隔，最後一人前加 "and" 字樣，其表示方法如Chen-Seng Huang, Chen-Chang Chen, and Chyr-Guan Chen。每一作者須附小註，以說明所屬機關單位與職稱，通訊作者附予星號" * "，並須附電子郵件信箱。題目與作者同置第一頁。

摘　　要：不分段落、宜簡潔明確。摘要之前應加作者、年代、題目、期刊、及卷期，摘要之後應附關鍵詞 (Key words)，關鍵詞不超過五個，每個英文關鍵詞第一字母應大寫。摘要以不超過五百字為原則。以中 (英) 文發表者須附英 (中) 文摘要，置於本文之後，且內容須與中 (英) 文摘要相符，並附通訊作者、作者之小註。

本　　文：依序為前言 (INTRODUCTION)、材料與方法 (MATERIALS AND METHODS)、結果 (RESULTS)、討論 (DISCUSSION)、結論 (CONCLUSIONS)、誌謝 (ACKNOWLEDGMENTS)、引用文獻 (REFERENCES)。除學名、專有名詞、化學式、數學公式、數字、度量衡單位及縮寫外，文句中不得中外文並用，需使用英 (外) 文以助明瞭時，須將英 (外) 文括號置於中文之後。材料與方法、結果兩部份如有必要須分段 (節)，每一段內並可再分小段，以助明瞭文稿內容。各段題目 (heading) 單行置於該段之前，小段題目 (subheading) 直接置於小段之首。如因結果繁複不易聯接討論，可

合併成'結果與討論 (RESULTS AND DISCUSSION)'。

表　格：

1. 每一表格應分別繕製，須以MS WORD®中 "表格" 工具製作，上方須有標題，以句點結尾，並按文中出現順序加上表**1. (Table 1.)**，表**2. (Table 2.)** 等冠號。
2. 中文稿之表格標題應中英文並列，冠號與表幅齊頭，表內項目及內容以英文書寫。英文標題及各欄英文細目，除第一字之第一字母大寫外，餘均小寫。單位使用SI units (如 g L^{-1}、Mg ha^{-1})，一律單數、英文字母。
3. 表格應加分析、簡化與歸納後始可列表。每一表格應在文中標示清楚，以便對照，且每一表格應單頁製作。
4. 表中內容如需說明，均須附加小註，以z, y, x,…等縮小小寫字母置於右上角，並於表下依次敘述。表格僅列橫線。星號"* (5%顯著性水準)"、"** (1%顯著性水準)"保留統計顯著性使用。
5. 性質類同之圖與表，務請精簡與合併。

圖　片：

1. 手繪圖版以黑色墨水於白色紙上分別繪製，掃描後存成JPG電子檔 (或JPEG電子檔) 上傳。如以電腦繪圖，力求清晰，亦存成JPG檔 (或JPEG檔) 上傳，或直接貼於本文之後。
2. 圖中所有標示應以英文書寫 (Arial字型) 或註明於標題之後。
3. 照片以光面紙為原則，清晰度以縮小50%可判讀為宜。多幅照片儘量製成併版；如有特別指明，應加圈或箭頭表明。顯微照相，應加比例尺大小；照片大小以不超過版面 (25 cm x 18 cm) 為宜，掃描後存成JPG檔 (或JPEG檔) 上傳，解析度300 dpi或以上，或直接貼於本文之後。
4. 圖版及照片應有標題，並按文中出現的順序加上圖**1.** (**Fig. 1**)、圖**2.** (**Fig. 2**) 等冠號，在文中須標示清楚，以便對照。中文稿標題應中英文併列，英文稿標題以英文書寫即可，力求明確。
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誌　謝：不分段落，包括人物、經費來源、報告之機關賦予編號等。誌謝可略去。

引用文獻：

1. 列舉確實曾引用之文獻。文獻以登載於學術性期刊、書籍、研討會之文章及學位論文為限 (不含推廣報告)，如引用網路學術性報告，須加註瀏覽、下載日期及網址路徑。文中涉及引用文獻時，根據該文獻的作者與年代加以括號：如 (Sung 1991)、(Lo 1991, 1992)、(Chen & Ho 1983)、(Sung 1991; Hsu *et al*. 1992)，以年代先後為序置於引用文處。
2. 文獻一律以英文撰寫，以姓名字母為序。作者相同時以年代先後為序。
3. 文獻之書寫方法以期刊為例，依序為作者、年代、題目、期刊、卷數、頁數。在每卷每期之頁碼都從數字 1開始的期刊，才必須加上期數。
4. 期刊名須依該期刊公告之縮寫撰寫；如無者，請參照Biosciences Information Service (1992) 出版之 "Serial Sources for the BIOSIS Previews Database" 或依該學術領域之慣例撰寫。
5. 文獻書寫範例如下：

(1) 原中文報告引用文獻出自學術性期刊

Hsu, S. H., T. Y. Chung, T. C. Kuo, and S. F. Wang. 1992. Studies on the micro-climate of greenhouse. I. Light environment of greenhouse. J. Agric. China 157:34–43. (in Chinese with English abstract)

(2) 原中文報告引用文獻出自著作性書籍

Sung, J. M. 1991. Nitrogen Metabolism of Rice. Chinese Agronomy Press. Taipei. 100 pp. (in Chinese)

(3) 原中文報告引用文獻出自編輯性書籍

Yang, C. M. and C. Y. Lin. 2003. Studies on rice precision farming system. p.1–12. *in*: Rice Precision Farming System. (Yang, C. M. and C. Y. Lin, eds.) Taiwan Agric. Res. Inst. Pub. No. 105. Taichung. 252 pp. (in Chinese with English abstract)

(4) 原中文報告引用文獻出自研討會專刊

Chang, C. A. 2001. Application and extension of virus-free seedling for the control of virus diseases of passion fruit in Taiwan. p.31–38. *in*: Proceeding of Symposium on Application of Healthy Plant for Control of Plant Diseases. November 24, 2001. Taichung, Taiwan.Taiwan Phytopathol. Soc. Publ., Taichung. (in Chinese with English abstract)

五、 單位、縮寫格式、括號及統計分析

1.凡文中出現之數值，除非另有特別之意義，一律用阿拉伯數字書寫。數字在句首之英文稿，改以文字表示。單位則用國際公認標準符號 (SI units) 代替全文，各常用之縮寫單位參照說明如下：

(1) 長度：公里 (km)、公尺 (=米) (m)、公分 (=厘米) (cm)、公釐 (=毫米) (mm)、微米 (μm)。

(2) 重量 (W)：公噸 (Mg)、公斤 (kg)、公克 (g)、毫克 (mg)、微克 (μg)。

(3) 容積 (V)：公升 (L)、公撮 (=毫升) (mL)、微升 (μL)。

(4) 面積：公頃 (ha)、平方公尺 (m^2)、平方公分 (cm^2)。

(5) 溫度：攝氏 (℃)、絕對溫度 (K，但須加以說明)。

(6) 時間：日 (天) (d)、時 (h)、分 (min)、秒 (s)。

(7) 濃度：莫耳 (M)、千分之一莫耳 (mM)、當量 (N)、千分之一當量 (mN)。

(8) 重力加速度：*g*。

(9) 分子量: kDa。

(10) 肥料三要素: 如以肥料種類$N-P_2O_5-K_2O$，如以元素N-P-K。

2.其他之縮寫：平均 (*avg.*)、大約 (*ca.*)、直徑 (diam.)。

3.括號：括號字型全以半形 ( )，括號前後各空一格。

4.統計分析：達顯著性水準以*、**或處理間顯著差異區分，以a、b、c、…表示者均應加註顯著機率值*P* (例如$P < 0.01$)；各統計量請採慣用符號，例如相關係數 (*r*) 、決定係數 (R^2)、變異係數 (cv)、標準差 (sd)、平均值標準差 (se) 等。

5.其他問題：

(1) 單位與其前面數值之間應空一格，如220 g。

(2) 數學運算符號與其前後數值之間應各空一格，如$P = 0.014$、23.6 ± 3.0、15 × 2。

(3) 在 ±、× 或to的前後兩數值若是含有單位，請用23.6% ± 3.0%、15 cm × 2 cm、20℃ to 30℃ (即勿省略前面數值之單位)。

(4) 數值起迄符號用– (半型破折號)，勿用 ~ 或 -，且與前後兩數值之間無須留空格，如3–6、2005–2007年、7月20–25日。

(5) 年代一律用西元。

(6) 濃度與容量單位使用mg L^{-1} (勿用ppm)。

(7) 轉速單位使用×*g* (勿用rpm)。

六、 其他注意事項

1. 本刊採網路線上投審，來稿請至本刊線上投審系統網址 (http://aspers.airiti.com/Aspers/WebHome.aspx)進行投稿作業，投稿前務請詳閱稿約及投稿須知，並可參閱最新一期報告。
2. 生物學名以斜體字表示，屬名第一字母大寫。學名於摘要、本文、每一圖表中第一次出現時，須寫全名，如*Citrus sinensis*；第二次出現時，屬名保留第一字母後加句點 “.”，種名全寫，如*C. sinensis*。生物學名於本文中首次出現時應加註命名者。
3. 中文稿件中，外文名詞應儘量翻譯成中文，若是不常見名詞應於文中第一次出現時以括號標出原文。摘要及本文中，英文縮寫於第一次出現時，須附全名。
4. 農作物品種名須加上單引號，中文用「 」(如水稻「台稉9號」、木瓜「台農2號」)，英文用‘’ (如rice ‘TK9’, papaya ‘TNG2’)。

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1. 稿件內容務須依照上列規定格式書寫及線上投稿，本期刊編審會保有修改與退稿之權利。文稿經本期刊接受後，由作者負責前二校。除筆誤與錯字外，作者在校稿時，不得擅自更改內容與數據。
2. 投稿前請詳閱稿約及參見最一期刊登報告，投審稿件程序請上網參閱本期刊網址：http://aspers.airiti.com/Aspers/webHome.aspx?jnliid=J0042。

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九、 其他未盡事宜本期刊得隨時修正或補充。

Instructions to Authors

Journal of Taiwan Agricultural Research (revised on 18 January 2013)

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Scope

After critical review and approval by the editorial board, JTAR publishes articles in plant science; biotechnology; agricultural chemistry; plant protection; agricultural engineering; agricultural economics and extension; industrial strategic management; biostatistics; and bioinformatics. Articles reporting experimentation or research findings in these fields will be peer-reviewed as research articles. Short articles (no more than 4 pages) concerned with experimental techniques, results, or unique phenomena will be peer-reviewed as short communications. Review articles will be under invitation by the editorial board of JTAR.

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See the latest issue of JTAR for style and layout. General rules are as follows:

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- At first listing both the common and chemical names of pesticides must be given.
- SI units must be used in the manuscript.
- Use the 24-h time system (e.g., 0200 and 1400) and abbreviate dates such as 3 October 2012 or 03/10/2012.
- Except when used with units, spell out numbers one through nine. Put commas for the decimal separator (e.g., 1,356,078 and 6,078) and place a zero before the decimal point (e.g., 0.6078 and 0.078).

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forms of .jpg files or .doc files or .docx files, one figure per file. Do not use word processing features such as automated bulleting and numbering, head and subhead formatting, or internal linking. Text should be typed in A4 size (29.6 cm long, 21 cm wide) in double-spaced and be numbered and aligned to the left side, while tables use single-spaced. Only one font and size (font no. 12) is used. Provide the document with an upper and lower margin of 3 cm and a left and right margin of 2 cm. Starting from the second page, indicate the page number on the lower right corner. When typing the manuscript, use the font of MingLiu (細明體) for Chinese and Times New Roman for English. Leave a single space between each English word and sentence.

The author submitting the manuscript should click on "Authors" on the front page of Journal's Online Submission and Review System. Under "Author" page, click "Submit a new manuscript". Confirm submission checklist and then complete the information related to the manuscript including a cover letter. Follow the sequence and instructions to fill out and upload the information. The system accepts only one corresponding author. If the manuscript has more than one corresponding author, indicate them in the cover letter. The remaining corresponding authors will be added to the manuscript when it has been accepted.

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Research Articles. Write a research article according to the mentioned scope in the following sequence: title, author(s), abstract, and text. Attach tables, figure captions, and figures at the end of text. In general, each manuscript should not exceed 10 printed pages.

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Author. List names of all authors below the title and place an asterisk after the corresponding author. The next sentence lists professional titles, institutions and complete addresses of all authors. Provide e-mail address of the corresponding author. Place title and author(s) on the same page.

Abstract. Each article has a single self-contained paragraph, containing the rationale, objectives, methods, results, and their meaning or scope of application specifically. Identify the important details to interpret the results but try not to exceed five hundred words. Do not cite references and avoid equations. In the next sentence, include no more than five key words and capitalize the first letter of each word. If the manuscript written in Chinese (English), place English (Chinese) abstract after the main text of the manuscript.

Text. The text includes the following sections in sequence: INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION, CONCLUSIONS (optional), ACKNOWLEDGMENTS (may be omitted), and REFERENCES. If required, combine the results and discussion section into "RESULTS AND DISCUSSION", and incorporate the conclusions into the discussion. If there is an appendix, place it before the references. Except necessary, do not mix English with other languages. If required, place headings and subheadings at the head of paragraphs of the sections MATERIALS AND METHODS and RESULTS.

Tables. Prepare tables with the tables feature of MS WORD®; do not use tab keys, space bars, or graphics boxes. Number tables consecutively with one table per page. Place tables at the end of text. Each datum needs to be contained in a defined cell. Table heads should be complete and self-contained but brief. All variables should be defined and abbreviations be spelled out in table footnotes. Follow the order of z, y, x, w, v, u,.... The *, **, and *** are used only to show statistical significance at the 0.05, 0.01, and 0.001 probability levels, respectively. Lack of significance indicates as NS. Asterisks or letters next to values indicate statistical significance and should align them in the same cell as the value. All footnotes should be set on separate lines. Capitalize only the first word in a row of the body of a table with the exception of proper nouns. Use only SI units (e.g., $\mu g\ L^{-1}$ and $Mg\ ha^{-1}$) in the singular forms.

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Acknowledgments. If required, provide it in a single paragraph. List institutional sponsors, the institutional article number, and contribution acknowledgment.

References. The reference section lists published literature and unpublished reports, theses, and dissertations cited in the manuscripts. Alphabetize the list by the surnames of the first author, followed by the other authors. Cite unpublished data, personal communications, and reports in the text only (in parentheses). Give the authors, year, complete article title, abbreviated journal title, volume number, and inclusive pages for journal articles. Give the authors, year, chapter title, pages, complete book title, book editor, publisher, and place of publication for book chapters. Add also the place and date of the conference for proceedings. For electronic references and others, see the current issues of JTAR. Single author references precede multiple-author references for the same first author. Use chronological order for references with identical authorship. Add a lowercase letter (i.e., a, b, c, etc.) to the year to identify same-year or multiple-author references for text citation. Write the author and year of the cited references inside parentheses; for example, (Chen & Ho 1983; Sung 1991; Hsu et al. 1992). Refer to the "Serial

Sources for the BIOSIS Previews Database" published by Biosciences Information Service (1992) for the abbreviations of an academic journal. Some reference examples:

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  Hsu, S. H., T. Y. Chung, T. C. Kuo, and S. F. Wang. 1992. Studies on the micro-climate of greenhouse. I. Light environment of greenhouse. J. Agric. Res. China 157:34–43. (in Chinese with English abstract)
- Reference from a book written in Chinese:
  Sung, J. M. 1991. Nitrogen Metabolism of Rice. Chinese Agronomy Press. Taipei. 100 pp. (in Chinese)
- Reference from an edited book written in Chinese:
  Yang, C. M. and C. Y. Lin. 2003. Studies on rice precision farming system. p.1–12. *in*: Rice Precision Farming System. (Yang, C. M. and C. Y. Lin, eds.) Taiwan Agric. Res. Inst. Pub. No. 105. Taichung. 252 pp. (in Chinese with English abstract)
- Reference from a proceedings written in Chinese:
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Units, Abbreviations, and Statistical Analysis

Use the SI system (Systéme International de Unités) in JTAR. Some commonly used abbreviated units:

- Length: kilometers (km), meter (m), centimeter (cm), millimeter (mm), micrometer (μm)
- Weight (W): ton (Mg), kilogram (kg), gram (g), milligram (mg), microgram (μg)
- Volume (V): litre (L), millilitre (mL), microlitre (μL)
- Surface: hectare (ha), square meter (m^2)
- Temperature: Celsius (℃), absolute temperature (K, but must be indicated)
- Time: day (d), hour (h), minute (min), second (s)
- Concentration: mole (M), millimole (mM), normal (N), millinormal (mN)
- Gravitational acceleration: *g*
- Molecular weight: kDa
- Three macronutrients in fertilizers: element—N-P-K ; equaivalent quantity—N-P_2O_5-K_2O

Other abbreviations:

- Average: *avg.*
- Approximate: *ca.*
- Diameter: diam.
- Concentration: e.g. mg L^{-1}.
- Frequency of a rotation: ×*g*.

Statistical analysis. Manuscripts should contain enough details of experimental design so that the results can be judged for validity and repeatability. The symbols * and ** are used to show differences in statistical significance. Add P value (e.g. $P < 0.01$) when a, b, c... are used to classify statistical

significance. Examples of commonly used symbols: *r* for correlation coefficient, R^2 for coefficient of determination, cv for coefficient of variation, sd for standard deviation, and se for standard error.

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